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THREE NEW SPECIES OF THE GENUS *METATHELAZIA* (NEMATODA)

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Skinker, 1931, found in the lungs of *Puma concolor hippolestes* and *Lynx rufus californicus* nematodes for which she proposed a new genus *Metathelazia* with *M. californica* as type species. Owing to the absence of bursa and rays in the male and the presence of genital papillae as well as some other morphological features, Skinker ascribed the genus *Metathelazia* to *Thelaziidae*. Dougherty (1943) recognized that the genus *Metathelazia* represents a member of STRONGYLATA and its genital papillae are homologues of the bursal rays which are typical of this group of nematodes. Dougherty placed *Metathelazia* in the subfamily FILAROIDINAE and established that the genera *Osleroides* Orlov, Davtian, and Lubimov, 1933, *Vogeloides* Orlov, Davtian and, Lubimov in Skrjabin, 1933, and *Pneumospirura* Wu & Hu, 1938, are synonyms of *Metathelazia*.

Five species of the genus *Metathelazia* have hitherto been described from lungs of various mammals. To this number three new species from Palestinian hosts may be added now. The comparative study of these species revealed some features the generic significance of which has not been sufficiently appreciated. When these features are taken into consideration the genus appears very homogeneous and the main difference is in the structure of the perioral region and in the number and disposition of male genital papillae. The variability of the perioral region, as revealed in the three Palestinian species studied in detail, represents a remarkable feature. In spite of the homogenicity of other anatomical details this region is different in all the mentioned species to an extent which usually is observed in members of different genera.

On the basis of new observations the following revised diagnosis of the genus *Metathelazia* is proposed:

Configuration of the perioral region shows a great variety: lip-like structures, buccal capsule and oral teeth may be present or absent; there are ten sensory perioral organs: two amphids and eight papillae disposed in four sublateral pairs. In each pair one papilla, which is larger, corresponds to the latero-dorsal or latero-ventral papilla (according to the terminology of Chitwood & Wehr, 1934); it lies a little posterior and lateral with respect to the smaller one which corresponds to the dorso-dorsal or ventro-ventral papilla. Oesophagus may be divided in two structurally different elements; anterior part of the intestine is rugose in some species or forms a number of short caeca-like diverticula which begin and end at different levels, peculiar to each species; the excretory system consists of two long wavy tubules which unite at the level of the

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anterior element of the oesophagus and open into a pyriform gland situated near the excretory pore; there are two small deirids. *Male:* no bursa; each species has a specific number of paired sessile papillae in addition to one unpaired papilla situated in front of the cloacal opening; spicules are bow- or sickle-shaped; gubernaculum consists of two separate elements. *Female:* vagina long, provided with a strong sphincter separating it from the vulva; the latter is situated near the anus which lies near the tip of the tail; ovoviviparous; ova with comparatively thick shell. Parasites of lungs of mammals. Type species *M. californica* Skinker, 1931.

In addition to the characters listed above, all species of the genus *Metathelazia* possess a peculiar organ which although seen and reproduced by various authors was misinterpreted by practically all of them. It is a loose membranous sheath enveloping the whole body of the nematode, attached to it only around the oral, excretory, anal and genital openings. This membrane, for which the term "teguminal sheath" is proposed, is peculiar not only for the genus *Metathelazia* but for all species of the METASTRONGYLOIDEA. It has been often mistaken for inflation of the cuticle or similar artifact, as a matter of fact it is a normal structure occurring in all specimens.

In feces of animals infected with two species of *Metathelazia* (*M. multipapillata* and *M. capsulata*), which were examined in a fresh state only eggs containing larvae but not free larvae were found. When the eggs were transferred into water or saline only a very small proportion of larvae hatched and these remained alive a few hours only. This suggests that these larvae do not hatch in natural conditions and they do not actively penetrate the intermediate host; apparently the larvae-containing eggs have to be swallowed by the latter. This may be true also for other species of the genus *Metathelazia*.

Metathelazia multipapillata sp. nov.
(Figs. 1–8)

Definitive hosts: Erinaceus roumanicus sacer (type host), *E. auritus.*
Location: Parenchyma of lungs.
Geographical distribution: Palestine, Syria.

This species was found in 16 out of 30 hedgehogs. It is imbedded in tubercle-like indurations 2–5 mm in diameter, usually in pairs, though sometimes several females may be present in one focus. The worms may easily be separated from the tissues and many whole specimens were secured.

Body long with respect to the width, enveloped with a wide teguminal sheath. The three-radiated oral opening is situated centrally, and is surrounded by six very low cuticular distentions. There are eight oral papillae: four small and four larger ones; each of the large papillae stands almost behind the corresponding small one, forming four sublateral pairs. Two large amphids are situated at the level of the large papillae. There is no buccal capsule. The oesophagus is indistinctly divided in two sausage-shaped, equally structured parts the posterior one being a little wider and longer. Intestine very wide at the start in comparison with the oesophagus; after an interval a little longer than the length of the oesophagus its wall forms irregular longitudinal rugae which disappear after some distance. There is a pair of small papillae-like deirids situated approximately at the level of the middle of the oesophagus; there is always a small difference in the situation level of the deirids. The excretory pore is situated ventrally at the same level. It leads into a small pyriform gland from which two narrow wavy tubules run along the inner wall of the body almost to its posterior extremity.

Male: Length 14–16 mm, maximum width 0.13–0.14 mm, length of the oesophagus 0.34–0.39 mm, maximum width 0.04–0.05 mm. Posterior extremity of the body forms a two-coiled spiral. Within the last coil there are 12 pairs of genital papillae in addition to one unpaired papilla just in front of the cloacal opening. The following is the disposition of the papillae: 5 pairs preanal, in two lateral rows; the outermost papillae are widely separated while the following ones are nearer each other; sixth pair adanal; the remaining papillae are postanal: the 7th and 8th pairs lie almost at the same level; the papillae of the 12th pair are different in structure from the other one, they apparently are phasmids. At the end of the tail there is a short conical appendage. Spicules sickle-shaped, they consist of a cylindrical outer stem and a thin inner edge, not reaching both extremities of the stem; the distal ends of the spicules have a small split. The spicules are almost equal in length which is 0.17–0.19 mm. Gubernaculum consists of two equal elements having the shape of an oval boat, 0.027–0.030 mm long.

Female: Length 30–40 mm, maximum width 0.19–0.26 mm. Oesophagus 0.34–0.42 mm,

maximum width 0.07–0.08 mm. Genital tubes fill up the whole body. Eggs oval, 33–35 × 47–49 μ, are enclosed in a thick shell, those near the vulva contain a developed larva. Vagina 2.4–3.0 mm long, thick-walled; there is a strong sphincter at its distal extremity. Vulva-anus distance 0.10–0.16 mm, anus-tip of the tail distance 0.06–0.07 mm. Tail conical.

The larvae hatched in artificial conditions are 0.290 mm long and 0.014 mm wide. Their tail is slightly bent ventrally and has no appendages.

Metathelazia capsulata sp. nov.
(Figs. 9–17)

Definitive hosts: Meles meles (type host), *Vulpes nilotica, Vormela peregusna.*
Location: Bronchi.
Geographical distribution: Palestine.

This species was found in 5 out of 7 badgers, in 3 out of 14 foxes and in 2 out of 8 marbled polecats.

Body short and thick, enveloped by a wide teguminal sheath. Oral opening is biscuit-shaped and is situated on the bottom of a deep depression lined up by a thick buccal capsule the walls of which form six bents thus making six lip-like folds arranged in threes on each lateral side. The central folds are large, the subventral and subdorsal ones are small. At the base of each fold inside the buccal capsule there is a small conical tooth. The teeth of the central folds coincide. There are four sublateral pairs of perioral papillae; in each pair the anterior smaller papilla is flat and transversely oval in cross-section, while the outer, larger one is semiglobular and protruding. There are two big amphids. Oesophagus is club-shaped and consists of two parts: anterior one of homogeneous structure, and posterior one containing glandular and muscular elements the latter being predominant; the posterior part of the oesophagus is twice as long as the anterior one. The nerve ring is situated just before the junction of the two parts of the oesophagus. The intestine is narrow at the junction with the oesophagus, it gradually becomes wider and at the widest level it gives off a number of large elongated caeca-like diverticula which thickly surround it for some distance. The arrangement and number of these caeca is subject to individual variations. There are two small knob-like deirids situated at the level of the nerve ring. Excretory pore is situated a little behind the level of the nerve ring. It is connected with a small oval gland which is associated with two narrow wavy tubules extending along the inner walls of the body almost up to its posterior extremity.

Male: Length 10–12 mm, maximum width 0.29–0.36 mm. Oesophagus 0.42–0.47 mm long and 0.07–0.09 mm wide. Posterior extremity makes up a two-coiled spiral; on the ventral side of the posterior coil there are 10 pairs of sessile genital papillae, in addition to an unpaired papilla situated in front of the cloacal opening. The following is the arrangement of the papillae: three pairs preanal, of these the anterior two pairs are largest; other papillae are postanal, of them three pairs of larger ones are situated near the edge of the body, three pairs of small ones form two rows near the median line and one pair of small conical papillae, which possibly are phasmids, is situated near the conical tip of the tail. In some specimens the preanal papillae are arranged asymmetrically. The spicules are almost equal in length, 0.31–0.34 mm, they are sickle-shaped and are composed of a stem and a thin edge along its inner curve. The gubernaculum consists of two equal elements each in the shape of an oval boat, of spongy consistency, 0.045–0.054 mm long.

Female: Length 18–20 mm, maximum width 0.44–0.47 mm. Oesophagus 0.52–0.55 mm long and 0.07–0.10 mm wide. In fully grown specimens the genital tubules fill up all the free space of the body. The vagina is 1.9–2.6 mm long, its walls are thick and it is provided with a strong sphincter at the junction with the vulva. Vulva and anus are situated near each other at the posterior extremity of the body; the distance vulva-anus is 0.14–0.16 mm, the distance anus-tip of the tail is 0.1 mm. Oval eggs have a thick shell, their size is 47 × 38 μ.

The first stage larva is 0.25–0.26 mm long and 0.014–0.015 mm wide. It is opaque and details of its structure may be recognized with difficulty. At the oral opening there is a minute nipple, it is followed by a short and thin duct which leads to the cylindrical oesophagus, which takes half of the body length; there is an excretory pore which is, however, seldom seen; no genital primordium or anus was observed; tail is abruptly conical, slightly bent ventrally and devoid of any appendages.

Metathelazia oesophagea sp. nov.
(Figs. 18–24)

Definitive host: Herpestes ichneumon.
Location: Bronchi.
Geographical distribution: Palestine.

This species was found in the bronchi of two out of three mongooses. Its name is chosen to emphasize the large oesophagus which is prominently larger than in any other known species of the genus *Metathelazia.*

Body relatively thick, enveloped by a teguminal sheath. The dorsoventrally oval oral opening is situated in a depression guarded by six lip-like projections; two lateral, and four sublateral ones which are lower. At the basis of each projection on the side facing the oral opening there is a small conical tooth. There are 8 sensory perioral papillae, all situated on the sublateral projections, in addition to two amphids which are situated on the lateral projections. Four of these papillae are large, semiglobular and protruding from the lateral sides of the perioral projections while four other are minute and may be seen with difficulty, they are situated on the ventral and dorsal slopes of the same projections.

The oesophagus is conspicuous by its size and in that it consists of two morphologically different elements: the anterior one is short and semitransparent while the posterior element is several times larger and consists of granular and fibrous elements. The nerve ring is situated a little anteriorly to the middle of the anterior part of the oesophagus. Thickly packed caeca-like diverticula surround the beginning of the intestine for a short distance, afterwards the intestine is smooth. The excretory opening is situated at or near the level of the middle of the anterior part of the oesophagus. It is followed by a small oval gland which unites two narrow wavy excretory tubules running along the inner walls of the body to almost its posterior extremity. Small semiglobular deirids are situated at the level of the excretory pore.

Male: Length 6.6–6.8 mm, maximum width 0.26–0.28 mm. Oesophagus 1.0–1.2 mm long, maximum width 0.19–0.21 mm. Distal extremity of the body forms a two-coiled spiral. On the inner side of the last coil there are 9 pairs of genital papillae in addition to an unpaired papilla situated in front of the cloacal opening. The arrangement of the papillae is as follows: first pair consisting of the largest papillae is preanal; the second pair is adanal; seven remaining pairs are postanal of these pairs the third and fourth are disposed in one transverse row, the outer papillae being larger than the inner ones; the 5th and 7th pairs are the smallest, while the papillae of the last pair are elongated (?phasmids). The tip of the tail has a small elongated appendage bent dorsally. The spicules are bent, thinner near their distal ends and are provided with a thin edge on their inner side; they are almost equal, 0.21–0.23 mm long. The gubernaculum consists of two equal boat-shaped elements of spongy consistency, 0.038–0.039 mm long.

Female: Length 7.8–8.2 mm, maximum width 0.34–0.37 mm. Oesophagus 1.23–1.27 mm long and 0.23–0.27 mm wide. Ovoviviparous. Size of the ova 40–46 × 30–35 μ. The thick-walled vagina is 1.0–1.1 mm long and it is provided with a strong sphincter at its distal end. The distance vulva-anus is 0.08–0.12 mm, the distance anus-tip of the tail is 0.1 mm. The tip of the tail is conical.

The following is the key to species of the genus *Metathelazia.*

1(8). Six conspicuous perioral lip-like projections 2

2(3). Oesophagus more than 1/7 of the length of the body; paired male papillae: 1 + 1 + 7 = 9*; distance vulva–anus = anus–tip of tail.
M. oesophagea sp. nov.

3(2). Oesophagus less than 1/10th of the length of the body 4

4(5). Distance vulva–anus is about twice the distance anus–tip of tail; paired male papillae: 3 + 1 + 3 = 7 or 4 + 3 = 7.
M. hainanensis (Wu & Hu, 1938) Dougherty, 1943

5(4). Distance vulva–anus = anus–tip of tail 6

6(7). Length of female more than 26 mm; length of vagina up to 7 mm; paired male papillae: 3 + 5 = 8.
M. massino (Davtian, 1933) Dougherty, 1943

7(6). Length of female less than 16 mm; length of vagina less than 1 mm; paired male papillae 4/7 + 4 = 8/11.
M. ascaroides (v. Linstow, 1879) Dougherty, 1943

8(1). Perioral projections inconspicuous or absent 9

9(10). Mouth capsule prominent; paired male papillae 3 + 7 = 10; distance vulva–anus twice as long as anus–tip of tail ..... *M. capsulata* sp. nov.

10(9). Mouth capsule absent or indistinct 11

* First number means preanal papillae, last one postanal papillae, the intermediate, if present, adanal papillae.

11(14). Posterior extremity of female conical 12
12(13). Paired male papillae 5 + 1 + 6 = 12 ..... *M. multipapillata* sp. nov.
13(12). Paired male papillae 4 + 3 = 7 ..... *M. californica* Skinker, 1931
14(11). Posterior extremity of female rounded; paired male papillae: 3 + 4 = 7. *M. felis* (Vogel, 1928) Dougherty, 1943

The systematic position of the genus *Metathelazia* appears to be uncertain. Some investigators ascribed it to SPIRUROIDEA while others to METASTRONGYLOIDEA. The latter view was especially supported by the latest papers of Dougherty. Indeed some characters suggest the proximity to METASTRONGYLOIDEA, namely: parasitism in the lungs, presence of the teguminal sheath, which is unknown in other groups of nematodes, the double gubernaculum which has many homologies in METASTRONGYLOIDEA. On the other hand the species of *Metathelazia* possess characters which are unknown in METASTRONGYLOIDEA: 8 perioral papillae as contrasted with 16 perioral papillae of METASTRONGYLOIDEA (except the genus *Dictyocaulus* in which only the ventrolaterals (sensu Chitwood & Wehr, 1943), are missing from the external circle), tendency for distinction of two parts of the oesophagus, unpaired male papilla. It is probable, therefore, that the genus *Metathelazia* will have to be separated from the METASTRONGYLOIDEA. This is, however, left for the future, when it is expected that the knowledge of the life history of the genus as well as the structure of other still unknown genera with similar anatomical peculiarities will elucidate its taxonomic position.

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Explanation of Plates

Plate I

Metathelazia multipapillata

Fig. 1. Perioral structure.
Fig. 2. Anterior extremity, ventral view.
Fig. 3. Anterior extremity, lateral view.
Fig. 4. Anterior extremity, low power magnification.
Fig. 5. Posterior extremity of male, ventral view.
Fig. 6. Posterior extremity of male, lateral view (only one spicule shown).
Fig. 7. Posterior extremity of female, low power magnification.
Fig. 8. Posterior extremity of female, high power magnification.

Plate II

Metathelazia capsulata

Fig. 9. Perioral structure.
Fig. 10. Anterior extremity, ventral view.
Fig. 11. Anterior extremity, lateral view.
Fig. 12. Anterior extremity, low power magnification.
Fig. 13. Posterior extremity of male, ventral view.
Fig. 14. Posterior extremity of male, lateral view (only one spicule shown).
Fig. 15. Posterior extremity of female, low power magnification.
Fig. 16. Posterior extremity of female, high power magnification.
Fig. 17. Egg and first stage larva.

Plate III

Metathelazia oesophagea

Fig. 18. Perioral structure.
Fig. 19. Anterior extremity, ventral view.
Fig. 20. Anterior extremity, lateral view.
Fig. 21. Anterior extremity, low power magnification.
Fig. 22. Posterior extremity of male, ventral view.
Fig. 23. Posterior extremity of male, lateral view (only one spicule shown).
Fig. 24. Posterior extremity of female.

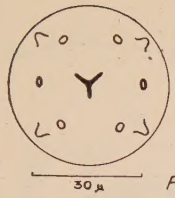

Fig. 1

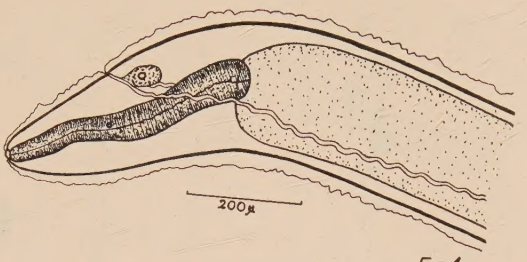

Fig. 4

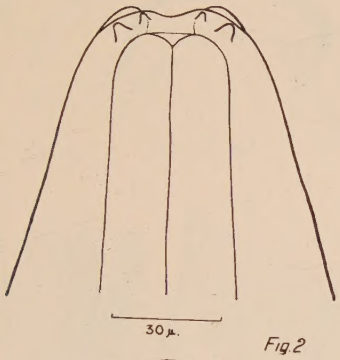

Fig. 2

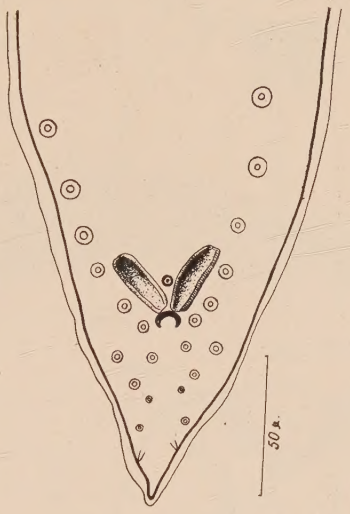

Fig. 5

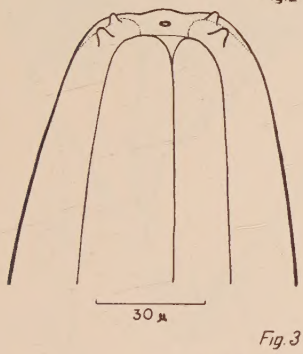

Fig. 3

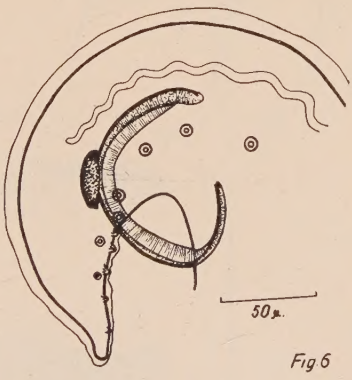

Fig. 6

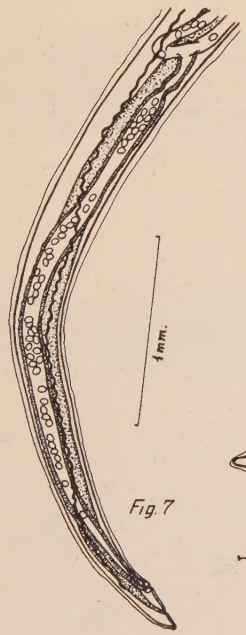

Fig. 7

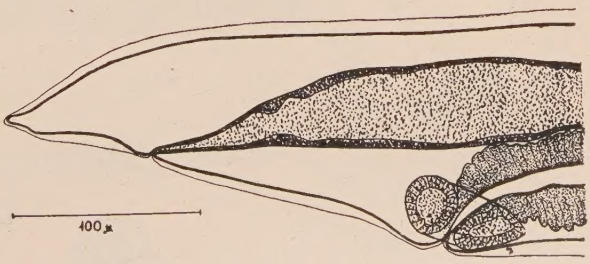

Fig. 8

PLATE I

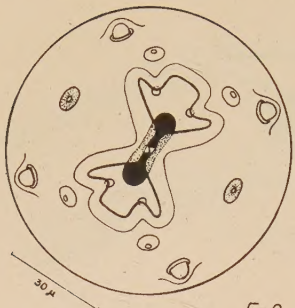

Fig. 9

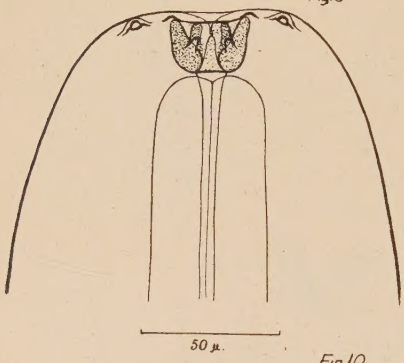

Fig. 10

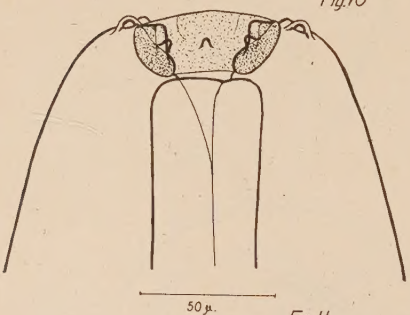

Fig. 11

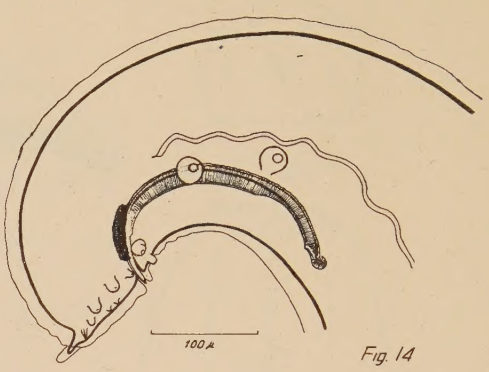

Fig. 14

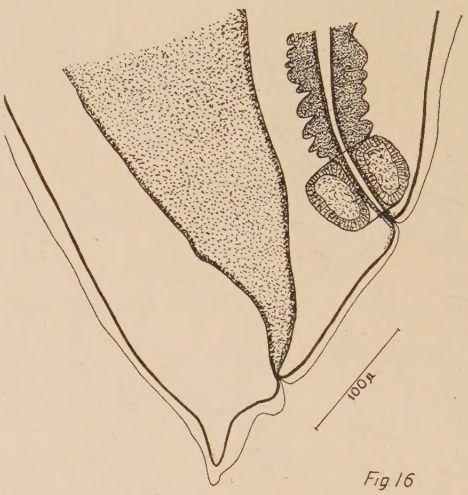

Fig. 16

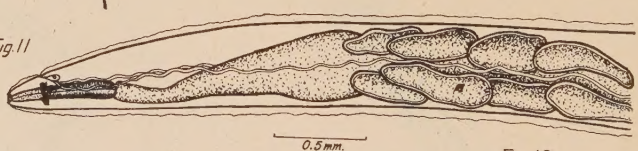

Fig. 12

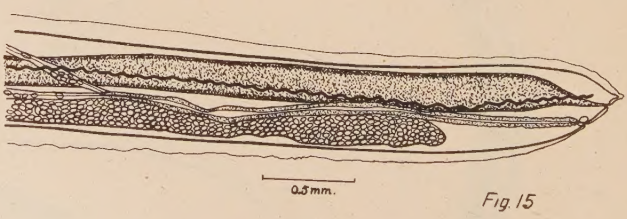

Fig. 15

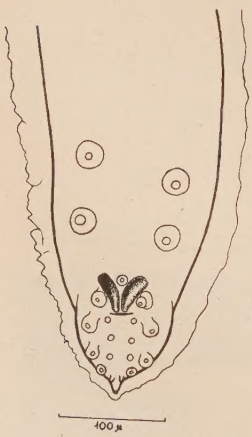

Fig. 13

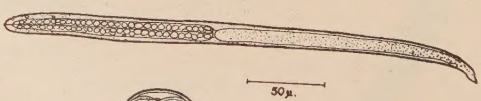

Fig. 17

PLATE II

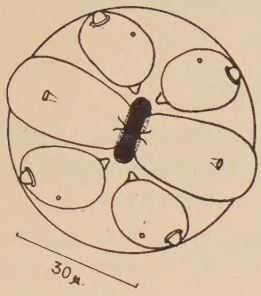

Fig. 18

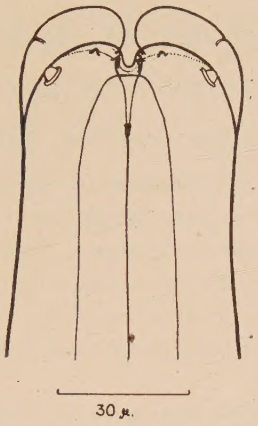

Fig. 19

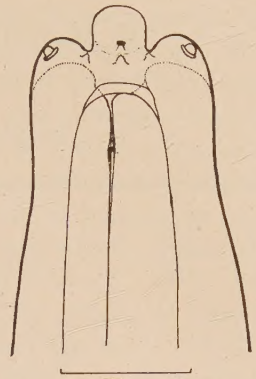

Fig. 20

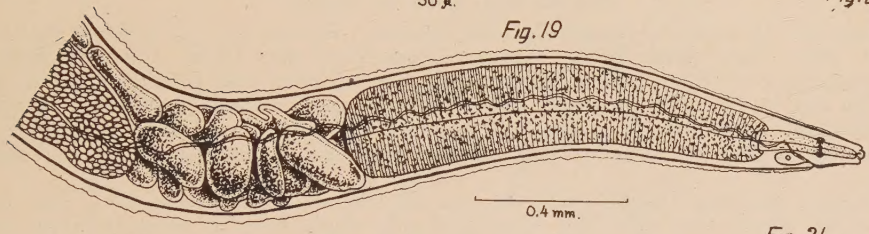

Fig. 21

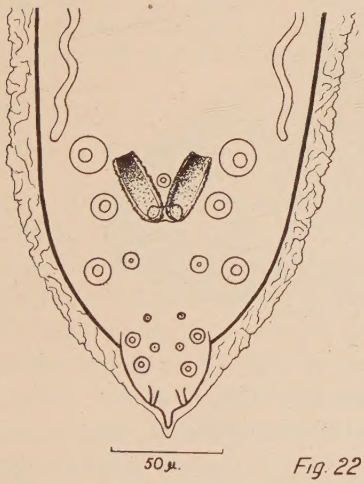

Fig. 22

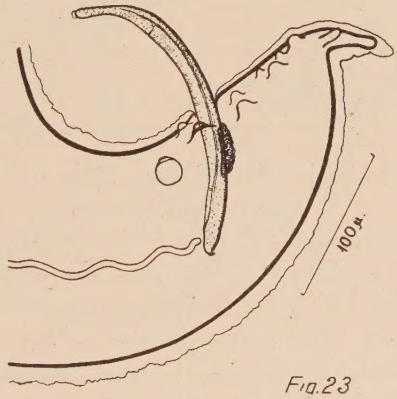

Fig. 23

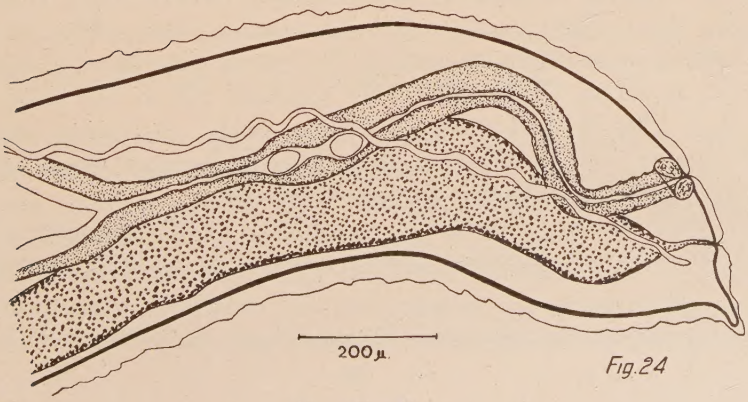

Fig. 24

PLATE III

A NEW ACANTHOCEPHALAN FROM THE BOB-WHITE

J. DAN WEBSTER

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In the course of examinations of Bob-white quail from Texas, three acanthocephalans were found in one bird and one in another. They represent a new species of the family GIGANTORHYNCHIDAE Hamann, 1892, genus *Mediorhynchus* Van Cleave, 1916. The quail viscera for examination were supplied by Mr. Valgene W. Lehmann; Dr. Asa C. Chandler directed the study. Dr. Harley J. Van Cleave and Dr. Donald V. Moore loaned specimens of other species from their collections for comparison.

Mediorhynchus colini new species

Diagnosis: Characters of the genus. Male unknown. Females 24 to 31 mm long by 0.6 to 1.0 mm wide. Body slender, ribbon-shaped. Pseudosegmentation slight. Cuticular vascular ducts prominent. Proboscis conical, 288 μ wide at base and 292 μ long, bearing 84 hooks, each 31 to 35 μ long (including the root) and arranged in twelve spiral rows of 7 hooks each. Counted in longitudinal rows, there are twenty-four rows of alternately 3 and 4 hooks. Spines on posterior part of proboscis 26 to 28 μ long, arranged in about forty longitudinal rows of 3 spines each.

Proboscis receptacle a double-walled muscular sac 1.08 to 1.25 mm long by 274 μ wide; both walls thicker dorsally than ventrally. Some of inverter muscle fibers of proboscis emerge from receptacle at posterior margin of outer sac. Retractor muscles of proboscis inserted with proboscis receptacle at boundary between hooks and spines. Lemnisci 3.48 to 3.56 mm long, each with 8 or 9 nuclei. Ovoid brain in center of anterior part of proboscis receptacle.

Nearly-mature eggs, in body cavity, with granular outer shell, 29 to 36 μ by 18 to 21 μ.

Host: Colinus virginianus texanus, Texas Bob-white.

Location: Jejunum.

Locality: Kleberg County, Texas.

Type: Mature female mounted on two slides, U. S. Nat. Mus. Helm. Coll. No. 36998.

Discussion.—There were three mature females, one lacking the anterior extremity, in a quail taken December 11, 1946, and a single mature female in a quail taken December 15, 1946. The present record represents the second occurrence of acanthocephalans in the Western Hemisphere in native galliform birds. (The first was by Leigh [1940]—*Mediorhynchus papillosus* in the Prairie Chicken in Illinois.) The closest relatives of *M. colini* are believed to be the following, with differential characters as stated: *Mediorhynchus otidis-houbarae* (Miescher, first published by Diesing, 1851) possesses a total of 15 to 20 hooks ranging in length up to 108 μ, according to Meyer (1933). *M. taeniatus* (Linstow, 1901) possesses 27 to 30 hooks attaining as much as 80 μ in length (Meyer, 1933, Dollfus, 1936). *M. numidae* (Baer, 1925) possesses 36 hooks 46 μ long (Baer, 1925). *M. giganteus* Meyer, 1931 possesses 72 hooks ranging up to more than 65 μ in length (Meyer, 1931). *M. turnixena* (Tubangui, 1933) possesses 104 to 140 hooks (evidently usually about 120) 55 to 65 μ long (Tubangui, 1933). As can be seen, *M. colini* possesses smaller hooks than any of the previously described species of this group and a larger number of hooks than any save *turnixena.*

Of the three species recognized by Van Cleave (1947) as found in the United States, *Mediorhynchus colini* most closely resembles *M. robustus* Van Cleave, 1916.

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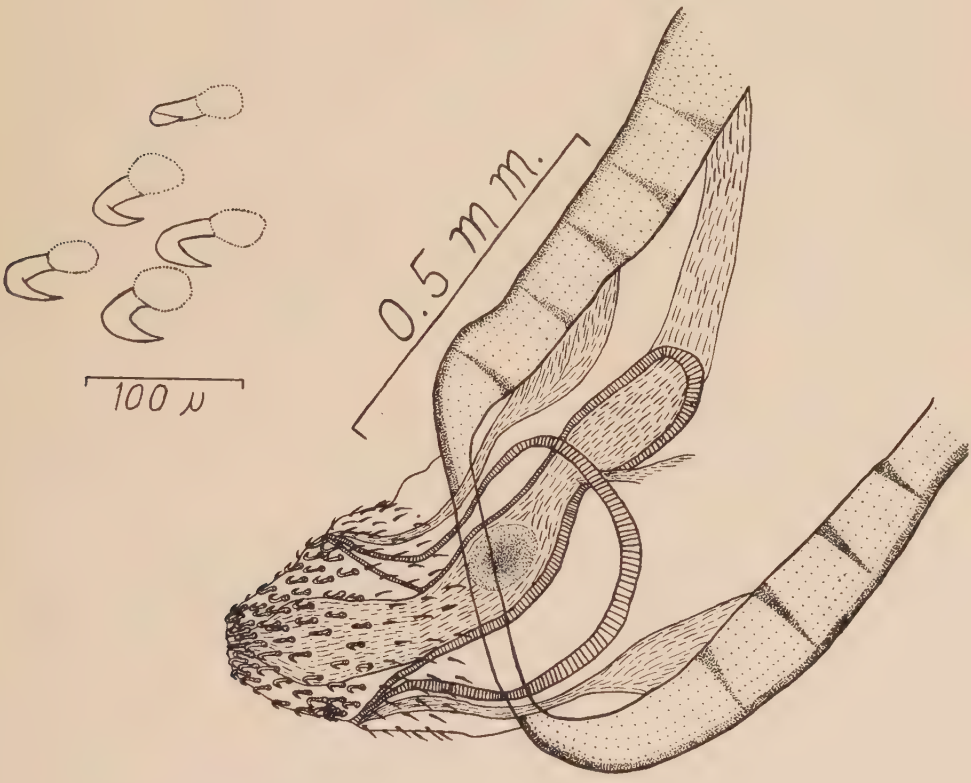
* A contribution from the Rice Institute, Houston, Texas.

Van Cleave does not state how many hooks are present in the latter species, but, judging from his figures, there are about 140. A specimen examined by the writer had 135 hooks. (Van Cleave gives [1947: 307] the number of longitudinal rows of hooks and the number of hooks per diagonal row.) *M. colini* possesses fewer and smaller hooks. (The measurement of the root of the hook as given by Van Cleave is the same as that of the hook as given by the writer.) In addition the two species differ in the structure of the proboscis receptacles.



Explanation of Plate

Both drawings are of the type specimen of *Mediorhynchus colini* n. sp., and were made with the aid of a camera lucida.

Upper. Details of several hooks from posterior part of anterior region of proboscis. The dotted portion is the root.

Lower. Anterior end of mature female, lateral view. The ovoid, stippled body is the brain. The lemnisci have been omitted. The nuscle at the posterior margin is the retractor of the receptacle. The stripes in the body wall are cuticular vascular ducts.

The writer is hesitant to assign the present species to *Mediorhynchus.* However, its closest relatives have been assigned to that genus by Van Cleave (1947). The writer at present lacks the facilities for a thorough investigation of the numerous, heterogenous species lumped into the one genus by Van Cleave. Van Cleave failed to explain the structure and function of the paraproboscideal sacs. He failed to examine specimens of *M. empodius* (Skrjabin, 1913), *M. giganteus* Meyer, 1931, and *Leiperacanthus gallinarum* Bhalerao, 1937, each of which served as the type

species or best-studied species of genera recognized by such authorities as Meyer (1933), Leiper (in Bhalerao, 1937), and Tubangui and Masilungan (1946). Yet Van Cleave stated (1947) that the genera *Empodius* Travassos (as constituted by Meyer, 1933) and *Leiperacanthus* Bhalerao were based on fundamental misconceptions of anatomy. The generic name *Empodius* is, apparently, invalid, as stated by Dollfus (1936) and Van Cleave (1947).

Van Cleave (1947) regarded host specificity as an "unwarranted belief" with regard to avian acanthocephalans. His tables of hosts in the same paper, however, cast some doubt on that attitude. The hosts of *Mediorhynchus grandis* are all birds of the suborder PASSERES, all but one of them of the family ICTERIDAE. For *Mediorhynchus papillosus* there are numerous records from various birds of the order PASSERIFORMES, plus one record from the GRUIFORMES and one (doubtfully indentified by Leigh [1940] and Van Cleave [1947: 310] from the GALLIFORMES. Of *Mediorhynchus robustus* there are numerous records from various species of passeriform birds, plus one record from the PICIFORMES. It is suggested that members of the genus *Mediorhynchus* show a strong tendency toward restriction to host orders. The validity of this suggestion is strengthened by an inspection of the host lists as given by Meyer (1933) for members of the family GIGANTORHYNCHIDAE.

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SYPHACIA EUTAMII N. SP. FROM THE LEAST CHIPMUNK, *EUTAMIAS MINIMUS,* WITH A KEY TO THE GENUS (NEMATODA: OXYURIDAE)*

JACK D. TINER[1]

A study of sciurid helminths from the University of Minnesota Division of Entomology Parasite Collection yielded a hitherto unnamed species of *Syphacia* which has been briefly discussed by Rausch and Tiner (1948). The description which follows is based on material collected from six individuals of the least chipmunk, *Eutamias minimus,* by S. Aldous at Grand Marais, Minn., September 23, 1938, and tentatively labeled *Wellcomia* sp. by Dr. A. B. Erickson. I am indebted to Dr. Erickson and Dr. C. E. Mickel of St. Paul, Minn., and to Dr. B. B. Morgan of the University of Wisconsin for aid in procuring these specimens. The worms were preserved in alcohol and glycerin, but most of them appeared to have been previously cleared in lactophenol. Several years' storage in cork-stoppered vials had caused all specimens to darken somewhat. They were cleared by the evaporation method and studied in glycerin. Addition of 1 part pure lactic acid to the glycerin temporarily removed the above-mentioned darkening and facilitated examination.

Two ventral cuticular bosses or mamelons were present on all undamaged males (Fig. 4); consequently, the species belongs in the genus *Syphacia* Seurat 1916.

Syphacia eutamii n. sp.
(Figs. 1 to 5)

Male: Length 2.07–2.56 mm; width 0.132–0.198 mm in middle of body. Esophagus, excluding bulb, 187–254 μ long by 30–40 μ wide just anterior to nerve ring and 36–63 μ near posterior end; bulb 66–94 μ long by 60–94 μ wide. Total length esophagus 244–348 μ. Nerve ring 105–115 μ and excretory pore 590–627 μ from anterior end. Most posterior of mamelons 98–165 μ in length and 539–660 μ from tip of tail. Second mamelon 99–165 μ long and 798–1,150 μ from tail tip. Almost half way from second mamelon to excretory pore cuticle occasionally somewhat thickened, but not projecting externally. Tail, including postanal cuticular projection, 69–102 μ long; cuticular projection, 33–50 μ long. Spicule 72–92 μ long and poorly chitinized except at base. Gubernaculum poorly chitinized, 21–26 μ long with barb consisting of a postero-dorsally directed process arising from each lateral margin (Figs. 3 and 5). Three pairs of caudal papillae present; one pair just anterior to cloaca, one just posterior to it, and one further behind on tips of projections which support small bursal alae.

Female: Length 6.4–9.9 mm; width 0.26–0.58 mm just posterior to vulva. Cervical cuticular inflation present, beginning at anterior end and extending about 300 μ posteriorly. Cervical papillae not observed. Lateral alae begin just behind cervical inflation and extend nearly to level of anus. Esophagus excluding bulb 346–396 μ long by 40–50 μ wide in anterior region and 66–83 μ wide at base; bulb 109–148 μ long by 115–148 μ wide. Total length of esophagus 472–538 μ. Nerve ring 115–165 μ, excretory pore 577–825 μ, and vulva 0.74–1.22 mm from anterior end. Tail 0.79–1.48 mm long. Ova 35–40 μ by 94–106 μ and somewhat flattened on one side.

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[1] Formerly Department of Veterinary Science, University of Wisconsin, Madison; present address 101 Vivarium Building, University of Illinois, Champaign, Ill.

Host: Eutamias minimus.
Location: Caecum and large intestine.
Locality: Grand Marais, Minnesota.
Type specimens: University of Minnesota, Division of Entomology Parasite Collection No. V–3204 (holotype male and allotype female), and U. S. National Museum Helminthological Collection No. 46304 (paratypes).

Syphacia eutamii is the only North American species in which the male is described as possessing two, instead of three, mamelons. Its poorly chitinized gubernaculum is a further feature serving to differentiate this species from the other North American forms, namely, *S. peromysci; S. obvelata,* and *S. thompsoni.* In respect to number of mamelons, *S. eutamii* resembles four African species (see: Sandground, 1939; Baylis, 1936a) which also are said to possess but two. However, *S. eutamii* differs from all four of these species. Of the four, *S. pallaryi* (Seurat, 1915) is apparently the most similar to it. The new species differs from *S. pallaryi* by having a shorter and otherwise dissimilar gubernaculum, as well as an esophagus which is shorter in proportion to body length. Only one more species, *S. sciuri* Mirza and Singh 1934, is described as having two ventral mamelons. However, this Indian species is said to have a third mamelon situated dorsally. It is the writer's opinion that this last structure could easily be an artifact. In any case, the demonstration of its presence or absence on more males from the same host species would be of much interest. Both males and gravid females are much smaller in *S. sciuri,* than in *S. eutamii.*

Faust (1929, 1939) and Tubangui (1931) stated that the male of the species *S. obvelata* may have two or three mamelons, but did not describe any variants possessing two. It would seem quite possible that their statement originated from the redescription of the genus by Yorke and Maplestone (1926).

The structure of the anterior end of the various species in the genus *Syphacia* is subject to considerable variation. In *S. obvelata* from *Microtus p. pennsylvanicus* Ord of Southern Michigan the four outer circle cephalic papillae are about 10 μ dorsal and ventral to the amphids when the latter are about 40 μ apart, and are located with the amphids on cuticular protrusions situated laterally (subventrally) on either side of the head. A third cuticular protrusion lies above the dorsal sector of the esophagus. By analogy with *S. thompsoni,* at least, these protrusions might be termed lips. The stoma of *S. obvelata* is roughly triangular at its anterior margin, and triquetrous for the short distance which the three sectors of the esophagus converge before becoming parallel. The lips of *S. thompsoni* cover the whole area of the head, and the cephalic papillae are located very near to the amphids which in this species are situated near the dorso-lateral margins of the subventral lips. Measurements on specimens of *S. thompsoni* from *Glaucomys sabrinus macrotis* Mearns collected by the writer and Dr. Robert Rausch in Michigan and Wisconsin indicate that the papillae lie about 10 μ postero-dorsal and postero-ventral to the openings of the amphids when the latter are about 96 μ apart. On *S. eutamii* the lips appear to be absent and the papillae are located relatively farther from the amphids, being about 13 μ away in a specimen in which the amphids were 40 μ apart.

The designation of the lips as dorsal and subventral in the above paragraph is not in accord with an illustration published by Price (1928) who pictured *S. thompsoni* with a ventral lip and two subdorsal lips. Furthermore, the terms

"ventral" and "subdorsal" have been applied to the "lips" of *"Syphacia"* by Chitwood and Chitwood (1938). A careful study of *en face* views and whole mounts of specimens of *S. thompsoni* reveals that there are dorsal and subventral sectors of the esophagus with well-developed lips corresponding to them.

A key has been prepared to facilitate differentiation of members of the genus. According to Erickson (1938), the male of *S. samorodini* was 0.5–0.7 mm long and the female 2.7–3.4 mm. This apparently served to differentiate it from *S. peromysci* Harkema 1936 in which the respective lengths of the two sexes were 0.91–1.3 mm and 2.0–2.5 mm (Harkema, 1936). Measurements of specimens of *Syphacia* collected from *Peromyscus maniculatus bairdii* (Hoy and Kennicott) in the University of Wisconsin Arboretum by Dr. Rausch revealed that the males ranged from 0.69 to 1.09 mm and the females from 2.27 to 3.21 mm in length. The distance which the mamelons projected from the body varied with the degree of curvature of the individual specimen. Since this Wisconsin material is easily identified to either species, it is the writer's opinion that *S. samorodini* may be considered a synonym of *S. peromysci*. *Syphacia baylisi* Maplestone and Bhaduri 1942 is regarded as a synonym of *S. muris* (Yamaguti, 1935). The specimens to which these names apply are from closely related hosts and there is nothing to indicate that they differ specifically (Baylis, 1936b; Maplestone and Bhaduri, 1942; Yamaguti, 1941). *Syphacia venteli* Travassos 1937 also may be indistinguishable from *S. muris,* but its occurrence in a different host genus makes its status more difficult to evaluate. *Syphacia trichosuri* Johnston and Mawson 1938 should be designated *Oxyuris* (*sensu latum*) *trichosuri,* since as was noted in its description, the vulva is located in the middle of the body, and there are three tooth-like structures at the base of the short mouth cavity. These differences, together with the fact that it occurs in a marsupial host, suggest that male specimens and the cephalic characters of *O. trichosuri* may be expected to differ widely from those of the genus *Syphacia.* Price (1928) pointed out that *S. stossichi* (Setti 1897) probably does not belong in *Syphacia.* Pending a redescription, the writer prefers to designate it as *Oxyuris* (*s.l.*) *stossichi.*

Syphacia ratti Roman 1945 and *S. frederici* Roman 1945 have been described on the basis of females alone, and are not included in the key. Like *S. baylisi* and *S. venteli, S. ratti* is as yet undifferentiated from *S. muris,* and may prove to be a synonym.

KEY TO SPECIES OF *Syphacia*

1. Males possess two ventral cuticular mamelons 2
   Males possess three ventral cuticular mamelons 7
2. Gubernaculum reaches maximum length of about 26 μ 3
   Gubernaculum exceeds 45 μ 4
3. Males about 1.3 mm long, gravid females 2.6–3.4 mm long.
   S. sciuri Mirza and Singh 1934.
   Males 2.0–2.6 mm long, gravid females 7.0–10 mm long.
   S. eutamii (this paper).
4. Spicule about 120–140 μ long 5
   Spicule less than 100 μ long 6
5. Gubernaculum barbed ..... *S. obubra* Baylis 1936.
   Gubernaculum not barbed ..... *S. pearsei* Baylis 1928.

6. Gubernaculum barbed ..... *S. pallaryi* (Seurat 1916).
   Gubernaculum not barbed ..... *S. paraxeri* Sandground 1933.
7. Male with six pointed processes on barb of gubernaculum, average length of females exceeds 7.5 mm ..... *S. thompsoni* Price 1928.
   Pointed processes on barb of gubernaculum absent, average length females less than 7.5 mm 8
8. Tail of female 850 to 950 μ long ..... *S. nigeriana* Baylis 1928.
   Tail of female does not exceed about 720 μ 9
9. Tail of male 30 μ or more in diameter just behind last pair of papillae at base of postanal cuticular projection.
   S. stroma (v. Linstow 1884) Morgan 1932.
   Tail of male narrows into a slender process less than 30 μ in diameter at base 10
10. Ova measure 110–142 $\mu \times$ 30–40 μ ..... *S. obvelata* (Rud. 1802).
    Ova less than 100 μ in length 11
11. Postanal cuticular projection of male about 35 μ or less in length.
    S. peromysci Harkema 1936.
    Postanal cuticular projection of male over 100 μ long 12
12. Distribution Brazil, host *Nectomys squamipes* ... *S. venteli* Travassos 1937.
    Distribution Japan and India, host *Rattus* spp. .. *S. muris* (Yamaguti 1935).

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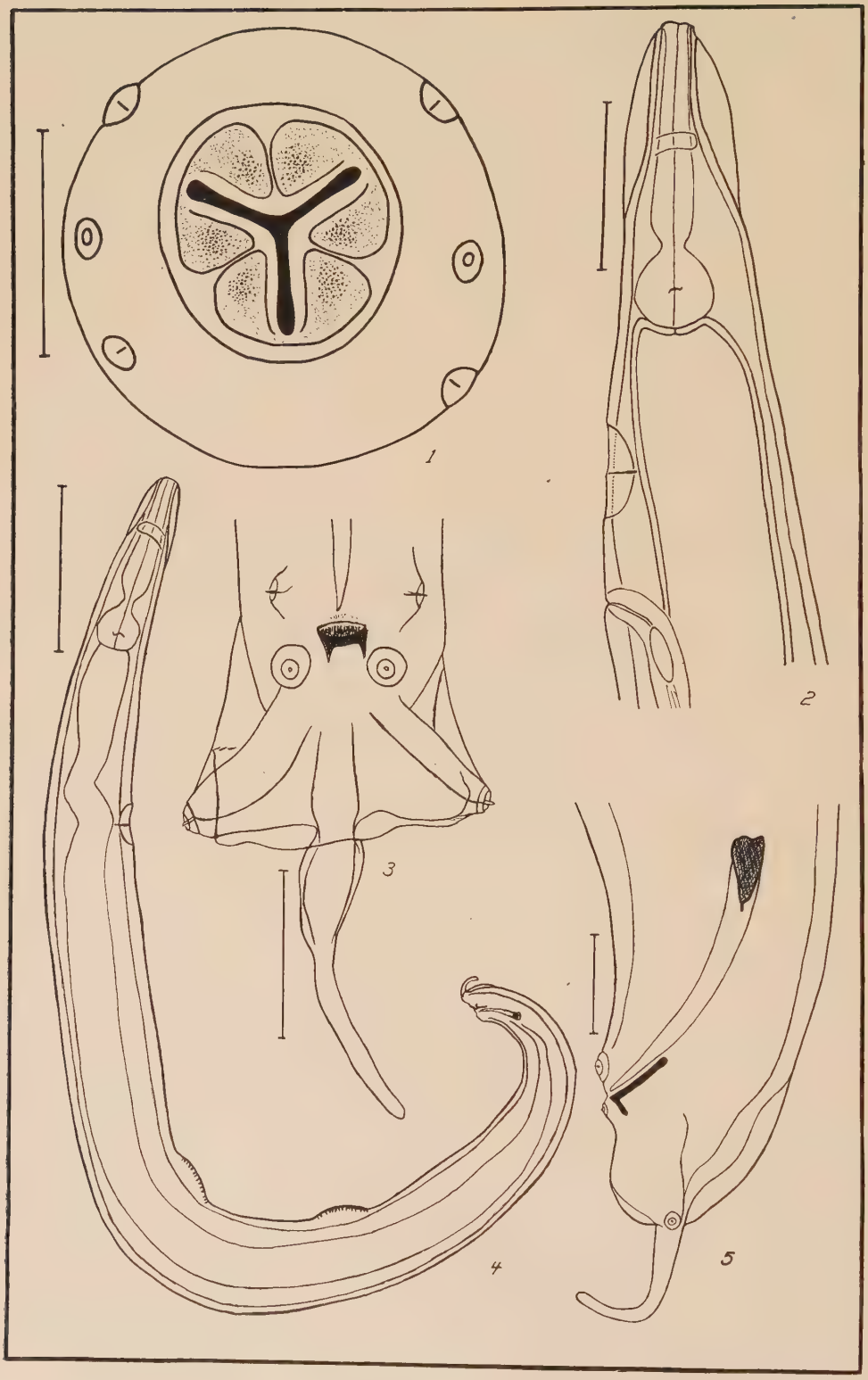
Explanation of Plate

Morphology of *Syphacia eutamii* n. sp. Scales for Figures 1, 3, and 5 represent 30 μ; for Figs. 2 and 4, 300 μ.

Fig. 1. *En face* view of female.
Fig. 2. Anterior end of female.
Fig. 3. Ventral view of male.
Fig. 4. Male.
Fig. 5. Lateral view of posterior extremity, male.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TWO CESTODES FROM A NIGHTHAWK

J. Dan Webster

Jamestown College, Jamestown, North Dakota*

A Nighthawk (*Chordeiles minor minor*) taken at Houston, Texas, on May 9, 1942, contained two species of cestodes, of which one is new and the other but little known. I want to thank Dr. Asa C. Chandler for his advice on the preparation of this manuscript and Mrs. W. H. Hake for her assistance in microscopical technique. Dr. E. W. Price courteously supplied specimens of *Amoebotaenia sphenoides* from the U. S. Bureau of Animal Industry collection for comparison. The new species is named in honor of the late Clarence J. Addis, who did much important work on the nutrition of tapeworms.

Bakererpes addisi new species

Diagnosis: Characters of the genus. Strobila 1.5 to 3 mm long, with no more than twelve segments. Scolex 206 to 304 μ broad; suckers 89 to 129 μ in longitudinal diameter; everted rostellum 58 to 78 μ in diameter; eight rostellar hooks in a single row, 81 to 91 μ long. Genital pores regularly alternate. Genital ducts pass laterally dorsal to longitudinal nerve and longitudinal excretory ducts.

Fifteen to eighteen testes in an irregular double row across the posterior part of the segment; vas deferens forms a coiled mass in the antero-medial part of the segment; cirrus pouch attains length of 292 to 379 μ and diameter of 58 to 69 μ; slender cirrus 128 to 182 μ long when everted, armed with numerous very minute spines.

Large multilobate ovary in center of segment; vitelline gland posterior to middle of ovary; ovoid Mehlis' gland antero-ventral to vitelline gland; seminal receptacle antero-poral to Mehlis' gland, communicating by a slender vagina with large, trumpet-like genital atrium. Inner part of genital atrium set with numerous short spines. Uterus at first a transverse tube dorsal to ovary, later forming large posterior lobes, finally half-filling the segment as a slightly-lobed sac. Spherical eggs, 38 to 40 μ in diameter, containing ellipsoid onchospheres, 31 to 33 μ by 26 to 27 μ.

Host: Chordeiles minor minor.

Location: Intestine.

Locality: Houston, Texas.

Type: U. S. Nat. Mus. Helm. Col.

Discussion: The above description is based upon eleven specimens mounted whole in balsam; all eleven were measured. Gross cross sections of one specimen were made with a razor blade and mounted in balsam.

Bakererpes addisi has larger and fewer hooks than the only other member of the genus, *B. fragilis* Rausch, 1947. Also, the ovary has several lobes in the former species, but only two in the latter. In the genus *Amoebotaenia* Cohn, the closest resemblance is to *A. fuhrmanni* Tseng Shen, 1932, which has ten hooks 70 μ long and cirrus pouch 44 to 52 μ long.

The above-described new species has been placed in the genus *Bakererpes* Rausch, in spite of several departures from the generic diagnosis as given by Rausch (1947). Most of these departures are regarded as specific differences. One difference is, however, important: In the description of *B. addisi,* above, the genital ducts are stated to pass dorsal to the longitudinal excretory vessels instead of between them. It seems probable that this difference is caused by an observational error on the part

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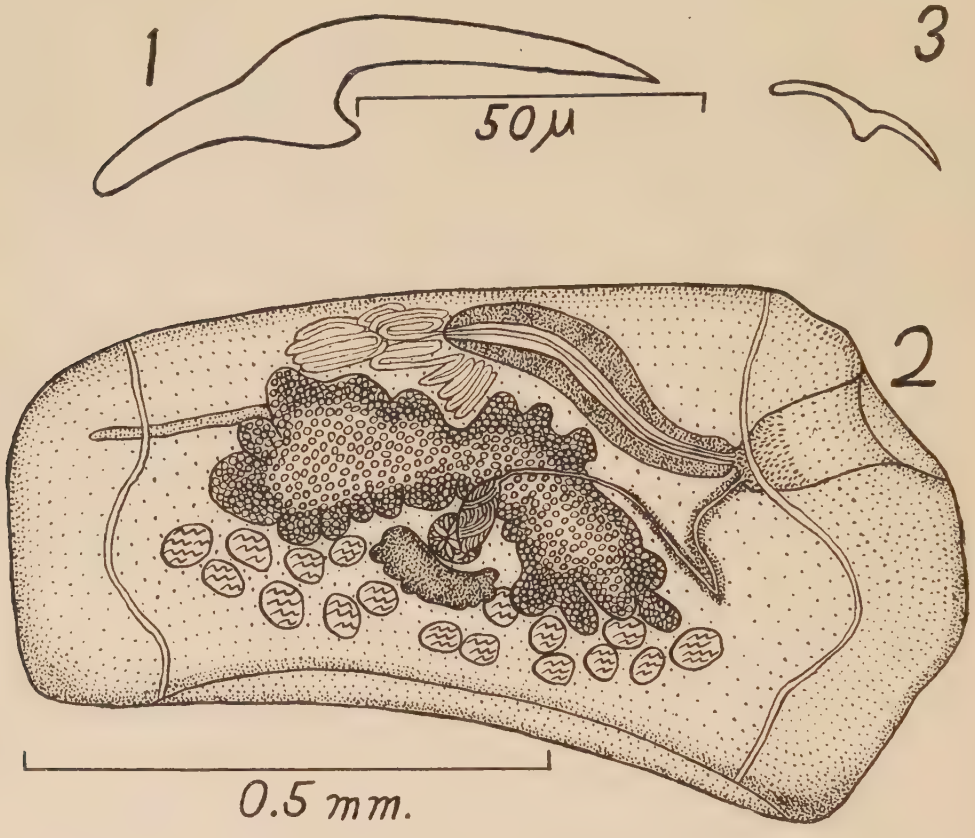
* A Contribution from the Rice Institute, Houston, Texas.

of either the writer or Dr. Rausch. This particular character is extremely difficult to determine in these minute worms, on account of the small size of the dorsal excretory vessel. Therefore, generic assignment has been made on the basis of more easily observed characters—particularly the shape and armature of the cirrus, cirrus pouch, and genital atrium.



Explanation of Plate

All figures were drawn with the aid of a camera lucida.

Fig. 1. *Bakererpes addisi* n. sp., rostellar hook.

Fig. 2. *Bakererpes addisi,* mature segment, ventral view. The uterus has begun to form and is visible in the upper left corner. Cirrus completely retracted.

Fig. 3. *Paricterotaenia pauciannulata* (Fuhrmann), rostellar hook. Drawn to same scale as figure 1.

In connection with identification of the above-described cestodes, the status of the genus *Amoebotaenia* Cohn was investigated. The relationship of the genital ducts to the longitudinal excretory vessels is ordinarily a generic character in cestodes of the family Dilepididae. In the genus *Amoebotaenia,* however, this character is in doubt or variable. The genotype is *A. sphenoides* (Railliet, 1892) Cohn, 1899, not *A. cuneata* (von Linstow, 1872) as given by some authors; the latter is a homonym of *Taenia cuneata* Batsch, 1782. In the poultry cestode, Yamaguti (1935) described the genital ducts as dorsal to the longitudinal excretory vessels. But Joyeux and Baer (1936) stated that the genital ducts passed between the excretory vessels.

Fuhrmann (1932) omitted this character from his generic diagnosis. The writer's observations on whole mount specimens and cross sections of *A. sphenoides* from chickens agree with those of Yamaguti. This character has been described for only three other species of the genus besides *A. sphenoides*. In *A. fuhrmanni* and *A. oligorchis* Yamaguti, 1935 (see Tseng Shen, 1932, and Yamaguti, 1935) the genital ducts pass dorsal to both excretory vessels. In *A. setosa* Burt, 1940, on the other hand, the genital ducts pass between the longitudinal excretory vessels (Burt, 1940). Another inconsistency in the genus is that *A. lumbrici* (Villot, 1883) Joyeux and Baer, 1939, possesses an internal seminal vesicle, a structure which is lacking in all the other members of the genus (Joyeux and Baer, 1939). It seems probable that *Amoebotaenia* as constituted by Tseng Shen (1932), Yamaguti (1935), Joyeux and Baer (1939) and Burt (1940) is an unnatural group which will eventually be subdivided into two or more genera.

Paricterotaenia pauciannulata (Fuhrmann, 1908) Fuhrmann, 1932

The present occurrence in *Chordeiles minor minor* seems to represent a new North American and host record. Previously this species was described from *Podager nacunda* in Brazil (Fuhrmann, 1908) and listed from *Caprimulgus europeae* in Europe (Fuhrmann, 1932). The material at hand consists of an entire specimen 3.5 mm long, with 31 segments, and a fragment of another worm. There is a single coronet of 18 rostellar hooks 24 μ long. (See figure 3.) There are 15 to 19 testes; the cirrus pouch is 164 to 184 μ by 20 to 22 μ.

Fuhrmann (1908) described the species as having 18 to 20 segments, 16 hooks 25 μ long, 18 testes, and cirrus pouch 200 μ long. His figure of the rostellar hook indicated a rather similar shape to that found in the present specimen, except that the guard was smaller. In view of the paucity of material from the Nighthawk, the above identification seems warranted.

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A MULTILOCULAR COENURUS OF *MULTICEPS* SP. IN *CAPROMYS PILORIDES* (SAY, 1822) DESMAREST, 1822

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Kourí and Angulo (1944) found a coenurus of *Multiceps serialis* in the conga hutia, *Capromys pilorides,* a native Cuban rodent. Pagenstecher (1877) reported a coenurus of *M. serialis* in the coypu, *Myocastor coypus,* a species belonging to the same family (Tate, 1935). Railliet and Mouquet (1919) found a coenurus, which they believed to be *Multiceps clavifer,* n. sp., in the coypu. Other completely identified coenuri have been recorded from a variety of hosts. Bonnal, Joyeux, and Bosch (1933), on the other hand, list several instances in which the species of coenuri could not be identified, i.e., for such hosts as *Cephalophus grimmi, Mus rattus* and *Cricetomys gambianus.* Taramelli and Dubois (1931), Roger, Sautet, and Paillas (1942), Cannon (1942), and Johnstone (quoted by Craig and Faust, 1943) reported the finding of coenuri in man, but the species could not be determined.

In this paper are reported the finding, in the conga hutia, of a coenurus whose identification to species could not be made, and the methods used in the attempt to identify it.

TAXONOMY AND HABITAT OF THE CONGA HUTIA

The conga hutia, *Capromys pilorides* (Say, 1822) Desmarest, 1822, is one of the four species of the genus living in Cuba and adjacent keys (Tate, 1935). Other members of the same sub-family (CAPROMYINAE) are found in Jamaica, Haiti, the Bahamas, and Venezuela. The sub-species of the host concerned in this report is *C. p. pilorides,* which is found over the entire island of Cuba. The other sub-species, *C. p. relictus,* is found on the nearby Isle of Pines.

DESCRIPTION OF THE COENURUS

The parasite was large, irregular, and composed of three masses embedded in the right rectus abdominis muscle; it projected into the subcutaneous tissue near the umbilicus, at the level of the right hypochondrium. Two of the masses were in the subcutaneous tissue and one was in the muscle. The larger one in the subcutaneous tissue was more superficial, almost spherical, pedunculate, and measured 2 cm in diameter; the smaller one was adherent to the deeper layers, flat, and covered an area of 1 cm^2. The largest of the three masses was in the muscle, and occupied an area of 5×4 cm. The two subcutaneous masses were connected with the large mass in the muscle by infundibula. Each of the two smaller masses consisted of a single cyst. The large mass in the muscle was composed of numerous small cysts embedded in a mesh of normal and reactional tissues (Fig. 1). When more than one cyst and diverticulum were present in a single cavity, they were frequently separated by a membranous wedge of reactional tissue.

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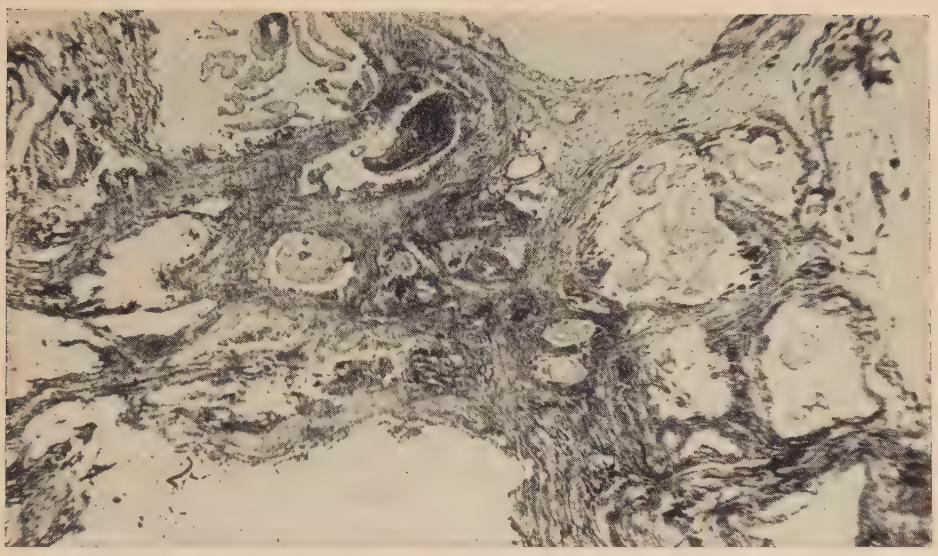

FIG. 1. Section of a portion of the main tumor-like mass. (Formalin: hematoxylin-phloxin-Orange G) (15×).

Most of the cysts showed diverticula and ovoidal or coral-like daughter cysts which were either detached or connected with the parent cysts by fine or thick peduncles (Fig. 2). Each cyst consisted of a thin, white, granular wall enclosing a transparent liquid. Some portions of the wall were denser and corresponded to invaginated diverticula. In very few instances could scolices be found (Fig. 3), and in one instance, two scolices were observed within a single cyst (Fig. 4).

FIG. 2. Cysts removed from cavities in the reactional membrane showing diverticula and daughter cysts.

FIG. 3. Cyst of Fig. 1 under higher magnification. The invaginated scolex shows the suckers and a few hooklets. (Formalin: hematoxylin-phloxin-Orange G) (250×).

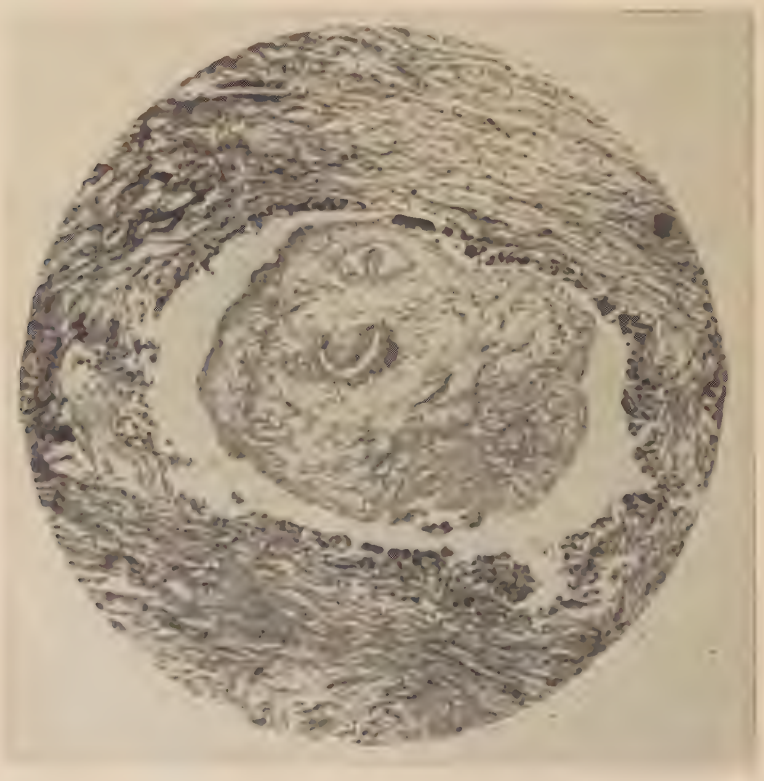

FIG. 4. Section showing a cyst surrounded by host tissue. Two, invaginated, incompletely developed scolices are seen within the cyst. Note the cellular infiltration around the parasite. (Formalin: hematoxylin-phloxin-Orange G) (130×).

DISCUSSION

The coenurus described above was distinguished from other cestode larvae by the following criteria: 1) The multilocular hydatid exhibits, characteristically, a laminated cuticular layer and the cyst content is usually gelatinous. The host is important since the multilocular hydatid has been observed only in ruminants (Dévé, 1912). 2) The alveolar hydatid is known to occur only in man (Dévé, 1912). This type of parasite, comparable to a cancer in its invasiveness of blood and lymph vessels and other tissues, lacks daughter cysts and a definite reactional membrane. 3) The unilocular hydatid exhibits typical size and shape. The histological features of the cyst wall are also characteristic. Scolices develop in brood capsules in the unilocular hydatid; in the coenurus, scolices arise directly from the cyst wall. 4) The so-called *Cysticercus racemosus,* although diverticulated, resides in a single cavity and can be removed as a whole; the parasite possesses one scolex; true daughter cysts are not present. The cyst is found only in the brain. 5) Spargana are solid, not cystic.

The coenurus described herein might be tentatively identified as *Multiceps serialis* (Gervais, 1845), Stiles and Stevenson, 1905, from the following characteristics: shape of the cysts, especially the presence of daughter cysts; type of host and host tissue in which the larva had developed; and locality in which the host was collected (a hutia parasitized with *M. serialis* was reported from the same locality by Kourí and Angulo, 1944). Clapham (1942), after a statistical study, concluded that *M. multiceps* and *M. serialis* are identical. The proper name of the species would, therefore, be *Multiceps multiceps* (Leske, 1870), Hall, 1910. The authors feel, however, that before species identification could be made, it would be necessary either to obtain measurements of the large hooklets or to note the typical grouping of the scolices in the cysts, which was impossible. Since this coenurus was composed of numerous small cysts in a honeycomb-like reactional membrane, the authors believe that it should be classified as a multilocular larva of *Multiceps* sp.

Other workers have described findings which are similar to some of those reported here, e.g., 1) Diverticula and daughter cysts of coenurus do not always contain scolices (Brumpt, Duvoir, and Sainton, 1934). 2) A coenurus of *M. serialis* has been reported to possess a peripheral reactional membrane, but with numerous diverticula in the wall (Bonnal, Joyeux, and Bosch, 1933).

SUMMARY

1. A coenurus of *Multiceps* sp. in *Capromys pilorides* (Say, 1822) Desmarest, 1822, is described. The morphology of the larva, especially its marked diverticulation and presence of daughter cysts, its development in the intermuscular connective tissue, the host species, and the locality in which the host specimen was collected, indicate its tentative identification as a larva of *Multiceps serialis.* It is felt, however, that further evidence is necessary before a definitive identification can be made.

2. The application of several criteria in the attempted identification of the species of *Multiceps* is discussed.

3. It is emphasized that the coenurus developed within a honeycomb-like

reactional membrane and that most of the cysts were sterile. The larva, therefore, is considered as multilocular.

ACKNOWLEDGMENT

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ON THE RECOVERY OF PROTOZOA AND EGGS OF SOME SPECIES OF HELMINTHS IN HUMAN FECES[1]

J. Bijlmer

From the Institute for Tropical Hygiene, Amsterdam[2]

In view of the fact that there was still a very unsatisfactory state of affairs in this field, an investigation was made for the most efficient technique for the diagnosis of protozoan infections of the human intestine. The possibilities which are at the disposal of the common practitioner were especially taken into account, although it goes without saying that also the clinics benefit by a simple but sufficiently accurate technique.

The following techniques can be considered:

1. Direct examination of feces;
2. Examination of feces after administration of a laxative;
3. The employment of a concentration technique.

For routine examination making fixed and stained films is not necessary and moreover is difficult and cumbersome. After an elaborate investigation on the cultivation of protozoa Brug (1936) came to the conclusion that this technique is unsuitable in practice.

Serologic investigation (Craig, 1944) is yet far from perfect, and intricate.

With duodenal drainages fewer infections with *Lamblia* are detected than with examination of feces.

For direct examination, extremely thin and adequately transparent suspensions of feces are made in a 2% eosin solution in water and in an iodine solution of 2% in 3% KI (2 I, 3 KI, 100 H_2O).

In the eosin film the living protozoa, trophozoites as well as cysts, stand out clearly as unstained particles against the red background, while the mobility is retained. Scrutinizing eosin films is easy and once used to it, one will always prefer eosin to physiological salt solution (Brug, 1936a).

No fat-containing laxatives may be used for the purging of patients because it would severely handicap the examination of the feces, but a saline purgative (e.g., 20 g $MgSO_4$), could be used. It is desirable that the intestine be quickly emptied and that the vegetative forms of protozoa be discharged alive.

While with purging the objective is to secure the vegetative forms, with concentration only the cysts are obtained. The vegetative forms do not survive such treatment.

In the Netherlands Bayer's technique was in vogue; since 1938 Faust's technique has come into use in America (Faust et al, 1938, 1939). Here in the Institute for Tropical Hygiene the latter technique has, since 1943, been tried out in a simplified form.

On 300 fecal specimens the direct examination and the three concentration techniques mentioned were applied side by side. (As a check, haematoxylin-stained films were also made.)

The procedure with the concentration techniques was as follows:

Bayer's technique:

1. Make in a mortar a suspension of 1 to 2 g feces in 10 cc tap water.
2. Add 10 cc of Bayer's fluid, agitate, let it settle down for a moment and pour off into a separator-funnel.

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[1] Condensation of a thesis. See bibliography.

[2] This investigation was made under the supervision of the late Professor Dr. S. L. Brug, to whom I am very much indebted for his wise advice and remarks.

3. Add 10 to 15 cc ether and agitate well for three minutes (now and then taking off the stopper); let it stand for 5 to 10 minutes and then drain the bottom layer of the fluid into a centrifuge tube.
4. Centrifuge for two minutes.
5. Pour off fluid and examine the sediment.

(Bayer's fluid: copper chloride, 2; copper acetate, 2; formol 100; aq. camphor, 500; distilled H_2O, 400; glacial acetic acid, 12.)

Faust's technique:

1. Make a suspension in a mortar of a fleck of feces the size of a pea in a few cc of tap water.
2. Filter this through a damp layer of cheesecloth into a centrifuge tube.
3. Add tap water till about half a centimeter from the top (proportion feces to tap water should be about 1:10).
4. Centrifuge for one minute at about 2500 RPM.
5. Pour off fluid and suspend sediment in freshly added tap water.
6. Recentrifuge for one minute.
7. Repeat the process until supernatant fluid is clear (3 to 5 times).
8. Suspend sediment in a 33% $ZnSO_4$ solution (specific gravity 1.18).
9. Centrifuge for two minutes.
10. Put on a slide one drop of iodine solution (as used with the direct examination); with a loop take something from the surface of the centrifuged material two or three times and mix with the drop of iodine. Place cover-glass on it and examine.

Simplified technique:

Suspend a fleck of feces of the size of a pea in a mortar with some 33% $ZnSO_4$ solution. If much detritus floats on the surface, filter through a layer of cheesecloth, but mostly this is not necessary and the fluid may at once be decanted into a centrifuge tube. Add 33% $ZnSO_4$ solution to half a centimeter from the top and centrifuge for two minutes, lift with a loop material from the surface and examine in iodine.

It proved to be an advantage to make the cover-glass rest with one side on the slide leaning against the drop and then to let it fall. In this way the greater part of the ova and cysts will be lying in the part of the film adjoining the side mentioned.

TABLE 1.—*Examination of 300 fecal specimens*

	Eosin iodine	Bayer	Faust	Simplified Faust	Number of infections
E. coli	73	96	99	98	104
E. histolytica	13	16	16	16	16
*E. tenuis**	8	13	12	12	13
"Iodine cysts"	20	14	22	23	24
E. nana	52	75	75	74	79
Lamblia	22	23	24	22	24
Chilomastix	23	34	33	32	36
Total	211	271	281	277	296

* *Entamoeba tenuis* is not considered here as a small race of *E. histolytica*, but as a separate species. There are obvious differences with *E. histolytica*. Thus we never find histolytic forms with *E. tenuis*, while the cysts generally contain much more and differently shaped chromatoidals than those of *E. histolytica*. It has not yet been proved that *E. tenuis* is pathogenic to man, but in any case it never causes an acute dysentery. To cats and dogs *E. tenuis* is much less pathogenic than *E. histolytica*. The size of trophozoites and cysts is 6–8 microns and always smaller than 10 microns.

In Table 1 the results are recorded. The fifth column gives the total number of infections which was shown by the whole series of manipulations. Now it appears that no one concentration technique detected all infections discovered. Except for the "iodine cysts" with which Bayer fails in nearly half of the cases, the results of the three concentration techniques are much the same. In those cases in which the stools contain only very few cysts—which does not imply at all that the infection is a light one—now one method yields success and then the other.

In Table 2 is recorded the percentage of gain which the various concentration techniques yielded compared with the direct examination. This proves that by concentration the number of positive findings is increased by about one-third, which is a rather important gain. With Bayer's technique the loss on the "iodine cysts" has

an unfavourable effect on the total profit. For this species of cysts Bayer's technique is completely unsuited; they are often deformed beyond recognition. If we eliminate the "iodine cysts," the figures become as is indicated in parentheses and the various techniques appear to be about equally efficient.

TABLE 2.—*Comparative findings of 300 specimens*

	Percentages of gain of the various techniques compared with the direct examination		
	Bayer	Faust	Simplified Faust
E. coli	31	36	34
E. histolytica	23	23	23
E. tenuis	63	50	50
"Iodine cysts"	..	10	15
E. nana	44	44	42
Lamblia	5	9	0
Chilomastix	48	43	39
Total	28	33	32
Total except "iodine cysts"	(35)	(36)	(33)

Finally the average concentrating rate of the three techniques was calculated (rounded off to full figures) of those cases for which it was possible. Now also the results appear to be not very divergent, although it is clear that the simplified technique concentrates somewhat less strongly than Faust's original method.

TABLE 3.—*Examination of 300 specimens*

	Averages of the concentrating rate of the various techniques			
	Bayer	Faust	Simplified Faust	Number of cases
E. coli	17×	×33	31×	51
E. histolytica	33×	×20	28×	11
E. tenuis	..	×68	..	3
"Iodine cysts"	..	×22	19×	15
E. nana	47×		27×	40
Lamblia	25×	×34	18×	21
Chilomastix	39×	×98	25×	16

Now if one has to make a choice Bayer's technique has the disadvantage of a separator funnel as an extra piece of required glass-ware, the three minutes of agitating well which is a very unpleasant occupation, and the duration of 20–25 minutes which it takes altogether. Faust's technique takes about as much time, while many manipulations have to be done with as many chances on pollution of the fingers. The simplified technique necessitates few manipulations and requires altogether only ten minutes—no longer than is necessary for making and examining a direct iodine film. As regards the direct iodine film the $ZnSO_4$ techniques have this other advantage that the structure of the cysts comes out more clearly and the shades are sharper and more differentiated. In my opinion therefore the simplified technique deserves to be preferred.

After this an investigation was made as to the value of purging. The stools of one hundred patients were examined once after application of a laxative (within two hours after defecation), and four times after spontaneous defecation on different days. In Table 4 are stated the results reached with the direct examination, with the simplified concentration technique (every time the previous findings were taken into account) and with the administration of a laxative (with the latter, besides an eosin film, a Delafield haematoxylin-stained film was also necessary, because otherwise the small species of amoebae could not be diagnosed).

TABLE 4.—*Results of a serial investigation of 100 persons*

	Number of infections after 1×		Number of infections after 2×		Number of infections after 3×		Number of infections after 4×		Laxative	Total number of infections
	Eosin iodine	Concentration	Eosin iodine	Concentration	Eosin iodine	Concentration	Eosin iodine	Concentration	Eosin Delafield	
Undifferentiated entamoebae	26	36	34	43	43	49	47	51	15 (2)	2
E. coli	2	4	3	5	4	6	5	6	24 (36)	51
E. histolytica	3	6	6	7	6	7	6	7	2 (3)	6
E. tenuis	2	2	2	2	3	3	3	3	7	7
Iodamoeba bütschlii	16	24	22	30	28	34	31	36	2	3
E. nana	2	3	3	3	3	3	4	4	32	37
Lamblia	6	10	10	13	12	14	12	15	2	4
Chilomastix									15	16
Total	57	85	80	103	99	116	108	122	84 (99)	126

In the column of the laxative there is a heading undifferentiated entamoebae. No less than fifteen times it could not be determined with certainty whether I had to do with *E. coli* or with *E. histolytica,* because I only consider as a certain distinguishing mark for the latter amoebae containing red blood corpuscles or the simultaneous presence of the characteristic cysts. (Many scientists are less exacting and rely also on nuclear structure, movement and formation of the pseudopodia, features on which, however, at most a probability diagnosis may be made.)[3]

By the findings of the related cysts by the other examinations of these fifteen cases, 12 proved to be *E. coli* and one *E. histolytica,* while in two cases also after four times still no cysts had been found (see figures in parentheses). This large number of undifferentiated entamoebae is in my opinion one of the objections to the laxative-techniques. In these cases the certainty is obtained that there are intestinal protozoa present but in order to make a diagnosis the search has to go on until cysts are found.

There was reason to suppose that with these four examinations and the examination after the administration of a laxative nearly all infections had been detected.[4] If, for convenience' sake, we assume that all infections had been located, it appears that: (1) a direct examination made once, detects about 45% of the infections; (2) an examination made once with a concentration-technique about 67%; (3) a direct examination made four times about 86%; (4) an examination made four times with a concentration-technique 97%; (5) an examination with the aid of a laxative about 78%, of which 11%, however, are uncertain as to species.

Dientamoeba fragilis and *Trichomonas hominis* have not been taken into consideration because they do not form cysts. The latter species was not found at all (is very rare in the Netherlands), the former one ten times after the laxative. (Brug (1936b) discovered that in this way only one third of these infections are detected, from which might be concluded that my cases had been infected for 30% with *D. fragilis*.)

All these fecal specimens were obtained from inhabitants of Amsterdam. Later the stools of 78 persons repatriated from Japanese camps were also put at my disposal and these showed a far higher percentage of *E. histolytica* infections. In Table 5 the final result of all examined cases is indicated with regard to the average concentrating rate of the various techniques.

From this study the following conclusions were drawn with regard to the protozoan investigations:

[3] It may be added that in the Netherlands and in the Netherlands Indies the hypothesis of Kuenen and Swellengrebel (1913) is accepted; this distinguishes three stages with *E. histolytica,* viz.: (1) the histolytic stage: large vegetative forms living as parasites in the wall of the intestine, which can be carried by the blood to various organs and which are able to consume red blood corpuscles. They reproduce by binary fission; (2) the minuta-stage: small vegetative forms which live as commensals in the intestinal lumen, do not phagocytize red blood corpuscles, and which resemble closely the vegetative forms of *E. coli.* They also reproduce by binary fission; (3) the cysts arising from the minuta-forms. Kuenen and Swellengrebel assumed that the minuta-stage is essential and the histolytic one incidental; in other words that a man can be infected with *E. histolytica* without experiencing injurious consequences.

[4] The stools obtained after purgation were also concentrated for cysts but these findings were not included in the tables in order not to make the material incongruous. The findings were about the same as with normally passed stools. For the examinations for cysts purgation or no purgation is apparently of no importance; only after a laxative proportionately more young forms are found. This fifth concentration yielded a gain of only one infection with *Endolimax nana.*

1. Of each fecal specimen an eosin-film is scrutinized. If the specimens are not formed, freshly passed stools are a primary condition; in other cases these are desirable. This film serves for the recovery of vegetative forms, *Lamblia* cysts, and for an orientating investigation.

TABLE 5.—*Results of all cases examined; concentrating rate*

	378 specimens			878 specimens	
	Bayer	Faust	Number of cases	Simplified Faust	Number of cases
E. coli	17×	37×	63	31×	126
E. histolytica	34×	35×	25	28×	32
E. tenuis	..	..	4	25×	10
"Iodine cysts"	..	22×	20	19×	26
E. nana	46×	39×	50	27×	97
Lamblia	25×	22×	24	18×	28
Chilomastix	39×	33×	17	24×	33

2. If the technique mentioned sub 1 should not lead to a definitive result and a sufficient number of cysts is present, an iodine-film is made.

3. If few or no cysts have been found a concentration is made with the simplified zinc-sulphate flotation technique.

4. If nothing should be detected the examination is repeated on following days until at least four examinations have been made.

5. Purging of a patient is mainly of interest for detecting *T. hominis* and *D. fragilis* because these two species do not form cysts.[5]

6. In looking for *Lamblia intestinalis* concentrating yields little result. For this the sub 1 and 4 mentioned points will suffice.[6]

Faust's zinc sulphate flotation technique besides being useful for protozoan cysts, is also suitable for some species of helminth's eggs. We could test this on the fecal specimens of the repatriated persons, which often contained *Ascaris,* Hookworm or *Trichuris* eggs and *Strongyloides* larvae. The direct method, Faust's (1938) technique and the simplified technique, Fülleborn's (1920), Hein's (1927) and Brug's (1921) technique were compared.

For the direct examination some feces are suspended in a drop of tap water and this is covered with a cover-glass. Fülleborn's technique is a brine flotation method: feces are suspended in a saturated brine solution (about 1-in-20 of fecal suspension); after half an hour, material is taken from the surface with a loop and examined.

With Hein's technique a dried fecal slide is covered with cedar-oil or liquid paraffin and then examined. Brug's technique is particularly suitable for *Strongyloides* larvae and consists of hanging the feces in a gauze in a pointed glass with lukewarm tap water. Sediment is examined after one to two hours.

The results are put forward in the Tables 6 and 7 and need almost no comment. The brine technique proves to be the best for hookworm, *Trichuris,* and fertilized *Ascaris* eggs, the dried slide for the unfertilized *Ascaris* eggs and Brug's technique for the *Strongyloides* larvae. The question may be raised whether or not a concentration technique is necessary for these eggs. *Ascaris* produces so many eggs that they can always be spotted in a well-prepared direct film, while for the unfertilized eggs the flotation techniques fail. *Trichuris* is of no interest for the clinics and we may accept that for hookworm infections of clinical significance there are so many worms present that the eggs can also be found in a direct film. Only for *Strongy-*

[5] For *Dientamoeba fragilis* and the flagellates except *Lamblia* cultures give excellent results.

[6] When *Lamblia* cysts are present in the feces their number is generally so large that they can be found in a direct film.

loides, which otherwise are not very pathogenic, a concentration technique would serve some purpose.

TABLE 6.—*Examination of 78 specimens of repatriated persons*

	H_2O	Faust	Simplified Faust	Fülleborn	Hein	Brug	Total
Ascaris fertilized ...	10	10	10	10	10	..	10
Ascaris unfertilized ..	3	1	1	..	3	..	3
Trichuris	3	8	16	18	14	..	22
Hookworm	14	25	26	26	17	..	27
Strongyloides	8	6	8	..	..	11	11
Taenia	2	..	..	1	2	..	2

TABLE 7.—*Examination of 78 specimens*

	Concentrating rate					
	Faust	Simplified Faust	Fülleborn	Hein	Brug	Number of cases
Ascaris fertilized ..	3×	5×	7×	6×	.....	10
Ascaris unfertilized	..	..	..	3×	.....	3
Trichuris	..	..	..	..	.....	..
Hookworm	7×	13×	24×	..	.....	12
Strongyloides	..	..	..	..	25–100×	7
Taenia	..	..	..	4×	.....	2

From the above follows the conclusion:

1. For *Ascaris lumbricoides, Ancylostoma duodenale* and *Necator americanus* an ordinary tap water film is sufficient; for *Ascaris* a dry slide may be made.

2. For *Strongyloides stercoralis* Brug's technique is recommended.

SUMMARY

An investigation was made of the value of Bayer's and Faust's concentration techniques and of the effect of fecal examination after the administration of a laxative for the examination for intestinal protozoa. It is concluded that in practice a simplified zinc sulphate flotation technique should be preferred.

By examining four times on different days (direct method and concentration) nearly all infections should be detected, not considering, however, *Trichomonas* and *D. fragilis.*

For hookworm, *Ascaris,* and *Trichuris* eggs a concentration technique is considered unnecessary; for *Strongyloides* larvae Brug's technique is recommended.

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A NEW SPECIES OF *PROSTHODENDRIUM* (TREMATODA: LECITHODENDRIIDAE) FROM THE LARGE BROWN BAT

AARON SEAMSTER AND ARTHUR L. STEVENS*

Examination of the intestine of a single large brown bat, *Eptesicus fuscus* (Beauvois), yielded ten specimens of an apparently new species of intestinal fluke of the genus *Prosthodendrium*. The material was fixed in Bouin's fluid, stained with precipitated borax carmine, and mounted in balsam. Measurements and figures were made from permanently mounted material. The following name is proposed for this species.

Prosthodendrium mizellei n. sp.

Fig. 1

Host and Locality: Eptesicus fuscus (Beauvois) Notre Dame, Ind.
Site of Infestation: Small intestine.
Specimens Studied: Ten.
Type Species: Cotypes, University of Notre Dame type collection, Notre Dame, Indiana.
Description: Body elliptical in outline (dorsal view) and 0.936 to 1.350 mm long by 0.396 to 0.666 mm wide. Integument aspinous. Oral sucker terminal, 0.072 mm long by 0.09 mm wide. Ventral sucker pre-equatorial; same size as the oral sucker; located entirely or nearly so, within the area circumscribed by the prostate mass. Pharynx somewhat indistinct in stained specimens and approximately 0.036 to 0.054 mm long by 0.036 to 0.054 mm wide. Esophagus of moderate length, 0.108 to 0.198 mm long by 0.027 to 0.036 mm wide. Intestinal crura pouch-like, extending to the anterior margin of the testes. Testes circular to oval in outline (dorsal view), located on either side of and somewhat posterior to the prostate mass; right testis slightly larger than the left, 0.205 mm long by 0.162 mm wide. Left testis 0.181 mm long by 0.147 mm wide. The prostate mass lies anterior to the testes and approximately equidistant from the lateral body surfaces. It is nearly circular in outline (dorsal view), measures from 0.126 to 0.198 mm in transverse diameter, and contains the coiled seminal vesicle. Transverse, sagittal and frontal sections failed to reveal the presence of a genital atrium. The genital pore opens dorsally and lies within the prostate mass near its anterior margin. Ovary more or less ovate in outline (dorsal view) with a tendency toward lobation in some specimens; 0.09 to 0.144 mm long by 0.09 to 0.126 mm wide, located adjacent and median to the right testis. The vitelline glands are bilaterally arranged and consist of numerous compact follicles which extend from the anterior margin of the testes up to about the level of the bifurcation of the intestinal crura. The uterus fills the entire posterior two thirds of the body and encroaches anteriorly upon the ovary and testes. Eggs elongate ovate in shape, 0.036 mm long, by 0.018 mm wide.

DISCUSSION

The position of the ventral sucker, lying entirely or nearly within the area circumscribed by the prostate mass, distinguishes this fluke from all of those so far described for the genus *Prosthodendrium*. Because of this characteristic, there is in reality no one species that can be considered as a close relative for a basis of comparison. However, in the description it can be noted that *Prosthodendrium mizellei* n. sp. exhibits various characteristics which separate it from other described species. The testes being considerably larger than the ventral sucker distinguishes it from *P. ascidia, P. chilostomum, P. longiforme, P. luzonicum* (Macy, 1936), *P. paeminosum* and *P. tetralobatum* (Cabellero, 1943), *P. transversum* (Byrd and Macy, 1942), *P. travassosi* (Macy, 1938) and *P. pyramidum* (Macy, 1936). The position of the ovary outside the acetabular region distinguishes it from *P. macnabi,*

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FIG. 1. *Prosthodendrium mizellei* n. sp. Camera lucida.

P. naviculum, and *P. swansoni* (Macy, 1936), from *P. scabrum* (Caballero, 1940), and *P. emollidum* (Caballero, 1943). The ovary being posterior to the ventral sucker distinguishes it from *P. buongerminii* (Lent et al, 1945) and *P. singularium* (Byrd and Macy, 1942), and *P. cordiforme* (Macy, 1936). The absence of oral spines separates it from *P. oraspinosa* (Macy, 1936). The ventral sucker being the same size as the oral sucker distinguishes it from *P. urna* and *P. dinanatum* (Macy, 1936), and the ovary being larger than the ventral sucker differentiates it from *P. lilliputianum* (Macy, 1936).

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TWO NEW DACTYLOGYRIDAE (TREMATODA: MONOGENEA) FROM THE GOLDEN SHINER*

AARON SEAMSTER

The family DACTYLOGYRIDAE Bychowsky, 1933, embraces four subfamilies, the BOTHITREMATINAE Price, 1936, the DACTYLOGYRINAE Bychowski, 1933, the DIPLECTANINAE Monticelli, 1903, and the TETRAONCHINAE Monticelli, 1903. The subfamily DACTYLOGYRINAE proposed by Bychowsky contained only the genus *Dactylogyrus* Deising, 1850, until Price (1938) proposed the genus *Neodactylogyrus*. Species possessing one haptoral bar were retained in the old genus *Dactylogyrus*, whereas those species with two haptoral bars were placed in the genus *Neodactylogyrus*. Kimpel (1939) considered *Neodactylogyrus* as valid and added six new species which he described from cyprinid fishes. Mizelle and Donahue (1944), however, rejected the genus *Neodactylogyrus* because: "the vestigal bar required for assignment of species, thereto, is differentially developed and not always visible in fixed specimens. . . ." It seems very probable that if a fission of the genus *Dactylogyrus* is to be made a more reliable character should be used.

This is the first report concerning gill parasites from the golden shiner [*Notemigonus crysoleucas auratus* (Rafinesque)] and contains new host and distribution records for members of the MONOGENEA. The material for the present work was collected in May 1939. The gills were prepared for examination according to the method used by Mizelle (1936), and the parasites were mounted in a glycerin-gelatin medium. All measurements and figures were made from permanently mounted material with the aid of a filar micrometer and a camera lucida.

Dactylogyrus parvicirrus n. sp.

Host and locality: Notemigonus crysoleucas auratus (Rafinesque); Culwell's pond, Warner, Oklahoma.

Site of Infestation: Gills.

Specimens Studied: Twelve.

Type Specimens: Cotypes, University of Notre Dame type collection, Notre Dame, Indiana.

Description: Small dactylogyrids 0.224 mm (0.180–0.270 mm) in length, greatest body width 0.063 mm (0.047–0.082 mm). Cephalic lobes poorly developed; eye spots four in number, usually of similar size and with members of individual pairs approximately the same distance apart. Posterior eye-spots slightly larger than anterior in only occasional specimens. Pharynx approximately circular in dorsal view and about 0.025 mm in diameter. Vitellaria dense, extending from pharyngeal region to the peduncle, and often obscuring the copulatory complex. Peduncle well developed; haptor hexagonal in shape and 0.056 mm (0.043–0.086 mm) long; greatest width 0.056 mm (0.034–0.081). Anchors situated dorsally on haptor, bases bifurcate, superficial roots longer than deep roots; anchor shafts solid and winged, points solid and unite with shaft to form an angle rather than an arc (Fig. 1). Anchor length 0.038 mm (0.036–0.041 mm), greatest width 0.012 mm (0.009–0.016 mm). Dorsal bar bent slightly in middle, about 0.030 mm (0.025–0.034 mm) in length (Fig. 2). Ventral bar not observed. Hooks large, each consisting of an elongate ovate base (side view), a solid shaft, a sickle-shaped termination and an opposable piece (Fig. 3). In most cases a posteriorly projecting process was observed arising from the sickle-shaped termination of the hooks. Hooks subequal in length with pairs numbers 1 and 5 being smaller; length of hooks of pairs 1 and 5 about 0.019 mm as compared with 0.024 mm for the remaining pairs. Copulatory complex small; cirrus and accessory piece basally articulate. Cirrus consisting on an enlarged basal portion and a simply curved shaft (Fig. 4); accessory

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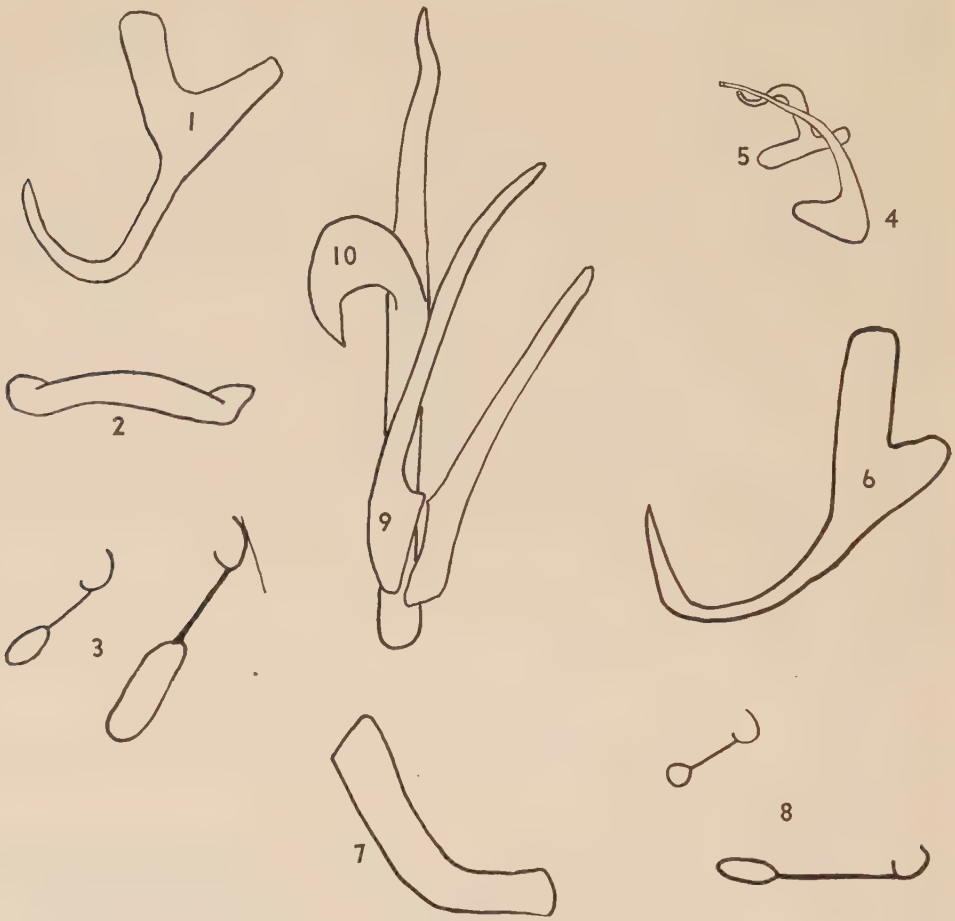
piece small, often "S" shaped, and arising from a large basal plate (Fig. 5). Cirrus 0.013 mm (0.011–0.016 mm) long. Accessory piece 0.008 mm (0.007–0.009 mm) long. Vagina not observed.

Dactylogyrus parvicirrus n. sp. is morphologically similar to *D. simplex* Mizelle, 1937 and *D. perlus* Mueller, 1938 but differs from them in (a) being smaller, (b)



FIGS. 1–5. *Dactylogyrus parvicirrus* n. sp. 1, anchor; 2, bar; 3, hooks; 4, cirrus; 5, accessory piece.

FIGS. 6–10. *Dactylogyrus aureus* n. sp. 6, anchor; 7, bar; 8, hooks; 9, cirrus; 10, accessory piece.

having an accessory piece without the characteristic knob-like projection, (c) having larger hooks, and (d) having a smaller copulatory complex.

Dactylogyrus aureus n. sp.

Host and Locality: Notemigonus crysoleucas auratus (Rafinesque); Culwell's pond, Warner, Oklahoma.

Site of Infestion: Gills.

Specimens Studied: Eight.

Type Specimens: Cotypes, University of Notre Dame type collection, Notre Dame, Indiana.

Description: Moderate-sized dactylogyrids with an average length of 0.320 mm (0.290–0.370 mm), greatest body width 0.054 mm (0.047–0.060 mm). Cephalic lobes poorly developed; eye spots four in number, approximately equal in size, and with members of individual pairs spaced

about the same distance apart. Pharynx subspherical and about 0.030 mm in transverse diameter. Vitellaria extending from pharyngeal region to peduncle. Haptor discoidal in shape; peduncle well developed. The single pair of anchors are situated dorsally on the haptor and are connected by a chitinous bar. Anchor bases bifurcate, superficial roots longer than deep roots. Shafts solid and winged; points solid and broadly recurved. Anchors 0.025 mm (0.021–0.027 mm) long and 0.018 mm (0.016–0.020 mm) in greatest width (Fig. 6). Dorsal bar 0.027 mm long, robust in appearance, bent slightly posteriad, ends rounded and slightly enlarged (Fig. 7). Ventral bar not observed. Hooks fourteen in number, subequal in size, and relatively small; those of pairs 1 and 5 about 0.011 mm in length as compared with 0.018 mm for the remaining pairs. Each hook consists of an ovate to elongate ovate base (side view), a solid shaft, a sickle-shaped termination, and a small opposable piece; posteriorly projecting process arising from the sickle-shaped termination of the hooks not observed in the specimens studied (Fig. 8). Copulatory complex large, cirrus and accessory articulated basally. Cirrus a slightly curved tube tapering distally to a point; length 0.046 mm (0.045–0.048 mm) (Fig. 9). Accessory piece long, tapering, and with a large hook-like projection arising in the middle third of its length; accessory piece 0.056 mm (0.054–0.061 mm) long (Fig. 10). A straight finger-like process 0.032 mm (0.030–0.036 mm) long arises from the basal part of the accessory piece. A large botuliform seminal vesicle was observed in some specimens. Vagina conspicuous, near middle left body margin; vestibule heavily chitinized.

Dactylogyrus aureus n. sp. resembles *D. intermedius* Wegner, 1909 but differs from it in (a) having a larger accessory piece with a broadly curved sickle-shaped termination, and (b) having a digitiform process arising from the basal part of the accessory piece.

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THE INHIBITION OF BACTERIA BY THE METABOLIC PRODUCTS OF TRICHOMONADS*

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Extensive research has been carried out to determine the inhibition of bacteria by the metabolic products of bacteria and molds. A survey of the literature revealed the production of an antibiotic by *Paramecium aurelia* (Sonneborn, Jacobson, and Dippell, 1946; Austin, 1946). The metabolic and disintegration products of certain strains of *P. aurelia,* when added to liquid cultures of other strains of *P. aurelia,* produced toxic and lethal effects. The action of known antibiotics on protozoa have been studied. In this study the Oxford cup plate assay method was used to determine the inhibition of bacteria by the metabolic products of trichomonads.

METHODS

The protozoa studied consisted of bacteria-free cultures of *Trichomonas foetus* and *Trichomonas vaginalis.* The *T. foetus* cultures, strains BR, O, and C, were obtained from Dr. B. B. Morgan, University of Wisconsin, Madison, Wisconsin. The *T. vaginalis* culture, No. 1, was provided by Dr. G. Johnson, Ortho Research Foundation, Raritan, New Jersey.

Schneider's citrate medium (Schneider, 1942) was used for culturing and assaying the three strains of *T. foetus.* The medium was modified by omitting the brom cresol purple.

The bacterial cultures were kept in stock on slants of the base agar (Schmidt and Moyer, 1944) and subcultural once a month. The seed inoculum bacterial cultures were subcultured daily from broth to broth (Schmidt and Moyer, 1944) and incubated at 37° C. Every two weeks the broth bacterial cultures were started from stock cultures. The bacteria chosen for this study, Table 1, were limited to those that would grow satisfactorily on the base agar, that would give even turbidity in the broth, and that were not too prolific growers in the 22-hour assay incubation period.

The Oxford cup plate assay method used for *T. foetus* is as follows:

Twenty milliliters of base agar (Schmidt and Moyer, 1944) were poured into Petri plates and left at room temperature 16–18 hours before seeding. The bacterial inoculum, consisting of 3 ml of base agar, seeded with a 1% 24-hour broth culture (Schmidt and Moyer, 1944) of a bacterium, was evenly distributed over the surface of the base agar. When the seed inoculum was firm, five sterile porcelain Oxford cups, warmed on a hot plate, were evenly arranged on each assay plate. The *T. foetus* cultures to be tested for bacterial inhibition were examined microscopically for sufficient growth of the trichomonads and tested for bacterial sterility in broth.

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Their pH was then determined with nitrazine paper and they were centrifuged at 1150 r.p.m. for 10 min. The supernatant fluid was decanted, thus removing the trichomonads from the protozoal culture material to be assayed. Four drops of the supernatant of the centrifuged protozoal culture material were placed in three of the cups by means of a sterile 1-ml pipette. Four drops of uninoculated control medium were added to the control cup. The control was treated in the same manner as the protozoan cultures, except the pH was adjusted to 5.0–5.5 with HCl. This pH adjustment was necessary as the *T. foetus* after incubation lowers the pH from 7.2–7.4 to 5.0–5.5. One cup, to which no fluid was added, served as a control on the heat of the cup. The assay plates were covered with sterile porcelain lids and incubated 22 hours at 37° C. The zones of inhibition were observed by reading over a substage microscope lamp and measured in millimeters. The surface of one of the zones of inhibition from each plate was touched with a sterile wire and seeded in broth to determine if the inhibitory substance was bactericidal or bacteriostatic.

The assay of each protozoan culture against one bacterium consisted of three plates with five cups per plate; three of these cups for the centrifuged protozoan culture material, one for the uninoculated, adjusted medium control, and one for a control on the heat of the cup. A complete assay consisted of three protozoan cultures of the same strain and same age against three bacteria, requiring a total of 27 plates. At least two complete assays were made with 7-, 10-, and 12-day *T. foetus* cultures, strains BR, O, and C, against the bacteria listed in Table 1.

The above procedure was modified with *T. vaginalis* by allowing the base agar to stand 12–14 hours at room temperature before seeding, centrifuging the protozoan cultures for 15 min. and assaying 2- to 3-day cultures against the bacteria mentioned later. Three complete assays were made with the *T. vaginalis* cultures against each bacterium.

Assays were also made with *T. foetus,* strain BR, 10-day cultures grown in Schneider's citrate medium without the citrate and brom cresol purple. *T. foetus,* strain BR, was maintained 8 weeks on this modified Schneider's citrate medium. Weekly transfers were made and subcultures incubated the first two days at 37° C and the remainder of the time until assayed at 22° C.

Quantitative citric acid determinations (Pucher, Vickery and Leavenworth, 1934) were made on pooled *T. foetus,* strain BR, 10-day cultures showing inhibition against *Salmonella pullorum* by the above-described assay method. An equal number of pooled tubes of uninoculated, unadjusted Schneider's citrate medium served as controls. The quantitative citric acid determinations were made on known volumes of the centrifuged trichomonad cultures and control as described in the cited reference, with the exception that filtration through Gooch crucibles was omitted and extractions were made in a Soxhlet apparatus using Whatman fat extraction thimbles, 33 × 94 mm. Two determinations were made on each sample of pooled protozoan culture material and on each pooled control.

RESULTS

Seven-, ten-, and twelve-day cultures of *T. foetus,* strains BR, O, and C, caused inhibition of *S. pullorum.* Seven-day cultures of strain BR showed inhibition of *Corynebacterium renale* and *Salmonella schottmuelleri.* The other bacteria listed

in Table 1 were not inhibited by 7-, 10-, or 12-day cultures of strains BR, O, and C, as determined by this method.

TABLE 1.—*Bacterial inhibition produced by* Trichomonas foetus. *Schneider's citrate medium*

Bacteria	Strains		
	Br	O	C
	7-day cultures		
Salmonella pullorum	+	+	+
S. typhimurium	–	–	–
S. schottmuelleri	+	–	–
Corynebacterium renale	+	–	–
Shigella gallinarum (M.S.C.)	–	–	–
	10-day cultures		
Salmonella pullorum	+	+	+
S. goettingen	–	–	–
S. gallinarum (Univ. of Kentucky)	–	–	–
S. choleraesuis	–	–	–
Staph. aureus 209	–	–	–
Aerobacter aerogenes	–	–	–
	12-day cultures		
Salmonella pullorum	+	+	+
S. paratyphi	–	–	–
S. enteritidis	–	–	–

+ Inhibition.
– No inhibition.

Table 2 shows the percentage of *T. foetus* assay cups showing no inhibition, fewer colonies or definite inhibition against *S. pullorum*. The maximum percentage of cups showing inhibition with strain BR were 12-day cultures and strain O, 10-day cultures. With strain C, the older the culture, the higher the percentage of inhibition. Attention should be called to the fact that the least number of cups (75) were used with 12-day cultures of strain C which gave the highest percentage of inhibition.

TABLE 2.—*Percentage of* T. foetus *assay cups showing inhibition of* S. pullorum. *Schneider's citrate medium.*

	Age of culture								
	7 days			10 days			12 days		
	Strains								
	BR	O	C	BR	O	C	BR	O	C
No inhibition	50	67	85	41	52	75	38	69	3
Fewer colonies	8	18	8	17	20	11	7	12	9
Inhibition	42	15	7	42	28	14	55	19	88
No. of cups	299	162	182	299	120	162	170	81	75

In assays totaling 150 cups, *T. foetus* strain BR, 7-day cultures, produced 10% fewer colonies and 10% inhibition of *C. renale*. Against *S. schottmuelleri*, the same strain and age cultures (54 cups) produced 24% fewer colonies and 9% inhibition. There is need for further study before evaluating these data.

The oldest cultures of all strains of *T. foetus* produced the largest zones of inhibition of *S. pullorum* (Table 3). Strain BR produced the maximum zones of inhibition of *S. pullorum* of these three strains using 7-, 10-, and 12-day cultures. The average zones of inhibition caused by strain BR, 7-day cultures, against *C. renale* were 4.3 mm and against *S. schottmuelleri* were 2.5 mm.

Ninety-five cups showing inhibition of *S. pullorum* by *T. foetus,* strains BR, O, and C, 7-, 10-, and 12-day cultures, were tested for bactericidal or bacteriostatic effects. Of those cups showing inhibition, 50% were shown to be bacteriostatic and 50% bactericidal when seeded in broth.

TABLE 3.—*Average zones of inhibition of* S. pullorum *by* T. foetus. *Schneider's citrate medium.*

Strains	Age of cultures		
	7 days	10 days	12 days
BR	6.2 mm	6.0 mm	7.9 mm
O	4.1 mm	4.9 mm	6.9 mm
C	5.0 mm	4.4 mm	5.8 mm

In the case of *T. foetus,* strain BR, assayed in Schneider's citrate medium, modified by omitting the sodium citrate, the percentage of cups showing inhibition (Table 4) increased with the age of the protozoan culture. These results compare favorably with the data in Table 2.

TABLE 4.—*Percentage of* T. foetus *assay cups showing inhibition of* S. pullorum. *Schneider's citrate medium without the citrate. Strain BR*

	Age of cultures		
	7 days	10 days	12 days
No inhibition	50.0	50.0	11.1
Fewer colonies	33.3	13.7	37.0
Inhibition	16.6	36.2	51.4
No. of cups	54	204	27

Of all the *T. foetus* cultures, grown in Schneider's citrate medium, inhibitory and non-inhibitory for *S. pullorum,* highest percentage of them showed a terminal pH of 5.5, with the exception of strain O, 12-day cultures, which showed the highest percentage at a terminal pH of 6.0. The largest percentage of cultures showing inhibition of *S. pullorum* had a pH of 5.5. The two exceptions were 7- and 12-day cultures of strain O, which showed the highest percentage of cultures with a pH of 6.0. The maximum percentage of *T. foetus* cultures, non-inhibitory for *S. pullorum,* had a pH of 5.5, with the exception of strain O, 10- and 12-day cultures. The variation of pH of inhibitory and non-inhibitory cultures is not of great enough significance to account for the inhibition of *S. pullorum.*

The pH of *T. foetus,* strain BR, 7-, 10-, and 12-day cultures, grown in Schneider's citrate medium modified by omitting the sodium citrate, inhibitory and non-inhibitory for *S. pullorum,* closely simulates the pH of the same strain and same age cultures grown in Schneider's citrate medium.

Considering the possibility that the inhibition of *S. pullorum* was due to the sensitivity of this bacterium to citric acid, quantitative citric acid determinations were made to determine the amount of citrate synthesized or utilized by *T. foetus,* strain BR, 10-day cultures showing inhibition of *S. pullorum.* Six determinations were performed and the average variation was an increase of 0.01 mg of citrate per ml of protozoan culture material which is negligible and within the accuracy of the method.

T. vaginalis, No. 1, 2-day cultures, grown in C.P.L.M. medium (Johnson and Trussell, 1943) produced no inhibition of *Salmonella schottmuelleri, S. typhimurium, S. pullorum, S. choleraesuis, S. gallinarum* (University of Kentucky), *S. paratyphi, S. enteritidis, Aerobacter aerogenes, Pseudomonas aeruginosa* and *Escherichia coli*

by the described assay method. Three-day cultures produced no inhibition of *Staphylococcus aureus* 209, *Staph. aureus* H, *Shigella gallinarum* (M.S.C.), *S. sonnei, S. paradysenteriae* Boyd 88, and *S. paradysenteriae* Flexner "V." *Streptococcus agalactiae* and *Eberthella typhosa* were unsuitable for assaying by this method as a white precipitate formed around each assay cup and the control cup.

Some substance or substances in cultures of *T. foetus* produced inhibition of *S. pullorum, C. renale,* and *S. schottmuelleri.* No attempt was made to determine the chemical nature of this material or to concentrate it. There is substantial evidence which indicates that it is not associated merely with the pH of the culture. The inhibitory substance tends to increase in the medium on cultivation whereas the pH of the medium is relatively stable at a pH of about 5.5 for the 7-, 10-, and 12-day cultures. Further, there is no relation between the pH of the culture and the production or non-production of the inhibitory substance. The mode of action of the material is not known since in different tests it has been bacteriostatic and bactericidal. This variation in results would probably indicate a difference in concentration of the inhibitory substance.

CONCLUSIONS

1. By the described Oxford cup plate assay method it was demonstrated that *T. foetus,* strains BR, O, and C, 7-, 10-, and 12-day cultures, produces inhibition of *S. pullorum.*

2. *T. foetus,* strain BR, 7-day cultures inhibited *C. renale* and *S. schottmuelleri.*

3. Sodium citrate is not necessary in the Schneider's citrate medium for the growth of *T. foetus* or for the production of substances inhibitory to *S. pullorum* by *T. foetus,* strain BR.

4. The pH of the *T. foetus* cultures was not responsible for the inhibition of *S. pullorum* in Schneider's citrate medium or in the medium modified by omitting the sodium citrate.

5. Citrate is not synthesized or utilized by *T. foetus,* strain BR, 10-day cultures.

6. The described assay method revealed no evidence of inhibition of bacterial growth caused by 2- and 3-day cultures of *T. vaginalis,* No. 1.

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WILD RABBITS AS RESERVOIR HOSTS OF THE COMMON LIVER FLUKE, *FASCIOLA HEPATICA,* IN SOUTHERN TEXAS

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Because of their wide distribution and susceptibility to the common liver fluke, *Fasciola hepatica,* wild rabbits serve as an important reservoir host for this parasite in regions where it is prevalent.

The importance of wild rabbits as reservoir hosts of the liver fluke has received little attention in this country. It is, therefore, the purpose of this article to review some of the significant literature on the rôle of wild rabbits as natural disseminators of the common liver fluke, and to report the results obtained from post-mortem examinations of a large number of jack rabbits, *Lepus californicus merriami* (Mearns) as listed by Miller (1924), and cottontails, *Sylvilagus floridanus* subsp. (vide Miller, 1924), collected in the Gulf Coast region of Texas.

REVIEW OF LITERATURE

Although the common liver fluke has long been known as the cause of a serious disease in sheep (Pitaillier, 1927), too little attention has been directed to wild rabbits and hares throughout the world as disseminators of this parasite.[1]

In Germany, according to Braun (1909), the first report of liver flukes in wild rabbits (*Lepus timidus*) was by Fromm in 1676, later by Zeder in 1800, and by Bremser in 1811; Diesing in 1850 confirmed Bremser's report. According to Braun (1909), Küchenmeister in 1855 omitted the hare as a host of the liver fluke in the first edition of his "Die in und an dem Körper des lebenden Menschen vorkommenden Parasiten" but included it in the second edition; likewise, Leuckart in 1863 omitted the hare as a host of liver flukes but included the domestic rabbit in "Die menschlichen Parasiten . . ."; he included the hare in "Die Parasiten des Menschen. . . ." Dewitz (1892) reported liver flukes from hares. Braun (1909) stated there were no liver flukes from hares in the parasite collection of the Zoological Museum in Berlin. He (1909) further stated that although many hares in the province suffered from a liver disease, no livers had been submitted for examination to determine what parasites were involved. Nöller (1925) stated that liver flukes were rarely found in hares in Germany, whereas Lerche (1933) reported that they were occasionally seen. Olt (1932) stated that, generally, liver flukes found in hares were sexually immature. More often evidence of infection rather than the flukes themselves was found.

In England, where an estimated 3,000,000 sheep died from fascioliasis in the epizootic of 1879–1880, Thomas (1883) stated that due to liver fluke infection the wild rabbit population in the vicinity of Oxford was practically decimated. He

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[1] Since submitting this manuscript the Standing Committee on Parasitology of the American Veterinary Medical Association stated "Wild reservoirs of infection are not thought to be of any practical importance, although rabbits may play a possible rôle in this regard." J. Am. Vet. Med. Assoc. **111**: 431. 1947.

found as many as 40 to 50 flukes per rabbit. It was his (1883) opinion that extermination of liver flukes would be impossible so long as rabbits and hares were present in the infested areas.

Pillers (1926) reported finding 22, 33, and 33 adult flukes, similar in appearance to *Fasciola hepatica,* in 3 rabbits collected from a region where sheep had not had access to the land for over 7 years. Moreover, fascioliasis had not been recognized in neighboring flocks. Although rabbits were frequently reported as being infected with flukes, examination of the livers failed to disclose them.

In France, Decroix (1873) and Martel (1909) observed clinical fascioliasis in wild hares.

In Italy, Perroncito (1916) observed wild hares infected with liver flukes.

In Japan, Kurisu (1931) found 1.56 per cent of the hares and 0.83 per cent of the rabbits infected with liver flukes.

In Australia, rabbits are recognized as playing a definite rôle as reservoir hosts of the liver fluke. McKay (1925) stated that in the Cooma district of New South Wales rabbits were found to be heavily infested with flukes. He observed, moreover, that in the control of liver flukes in cattle and sheep, it is important to take into consideration the rôle of the rabbit as a disseminator of these parasites to domestic ruminants.

In the United States, Dikmans (1930) collected 2 specimens of *F. hepatica* from the bile ducts of a cottontail, *Sylvilagus floridanus alcer,* in southern Louisiana. Krull (1933) infected experimentally 2 laboratory-raised cottontails, *S. f. mallurus.* From one rabbit examined 20 days after infection, he recovered 25 immature liver flukes. Numerous liver fluke eggs were found in the feces of the other rabbit about 3 months after infection, and from which 3 mature flukes were found on post-mortem examination. Olsen (1947) reported jack rabbits, *Lepus californicus merriami,* and *Sylvilagus* sp. naturally infected with liver flukes in southern Texas.

MATERIAL AND METHODS

Jack rabbits and cottontails were collected at intervals throughout the course of a year on pastures where cattle ranged. The livers were removed and preserved in formalin as soon as the rabbits were collected.

Inspection of the livers was done in the laboratory by (1) microscopic examination of the contents of the gall bladder for eggs of the fluke, and (2) thinly slicing the livers and looking for the parasites.

RESULTS OF EXAMINATIONS

Of the 309 jack rabbits examined from Refugio County, 99, or 32 per cent, were infected with liver flukes. Of these infected hares, 56, or 56.6 per cent, had flukes in the liver, and 43, or 43.6 per cent, had only eggs of flukes in the gall bladder, no flukes being found. There were 4 instances in which immature flukes were found along with mature ones. The number of flukes per hare ranged from 1 to 7. The incidence of infection in the jack rabbits remained high throughout the entire year. The results of these examinations are summarized in Table 1.

Of the 24 cottontails collected from the pastures where the jack rabbits were taken, 5, or 20.8 per cent, were positive for liver flukes. One of these animals har-

TABLE 1.—*Incidence of infection among wild rabbits from one ranch in the coastal prairie of Texas with the common liver fluke,* Fasciola hepatica

Date	Rabbits—		Rabbits infected—												
	Examined	Uninfected	With eggs only in gall bladder	With flukes of all sizes	Total	Per cent	With gravid flukes in liver	With non-gravid flukes in liver	With indicated number of flukes in each						
									1	2	3	4	5	6	7
Jack rabbits (*Lepus californicus merriami*)															
5 24–46	108	68	18	22	40	37	22	4	10	8	2	1	0	1	0
8–5–46	76	56	12	8	20	26.3	8	0	4	3	1	0	0	0	0
10–3–47	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0
11 23–46	5	4	1	0	1	20	0	0	0	0	0	0	0	0	0
1–27–47	12	9	2	1	3	25	1	0	0	1	0	0	0	0	0
3–3–47	21	17	1	3	4	19	3	0	1	0	1	1	0	0	0
4 15–47	8	6	1	1	2	25	1	0	0	0	0	1	0	0	0
5–16–47	38	27	5	6	11	29	6	0	3	1	1	0	0	1	0
6–7–47	14	9	0	5	5	32	5	0	1	1	0	0	0	1	2
8–8–47	23	10	3	10	13	43.5	10	0	4	3	2	1	0	0	0
Total	309	210	43	56	99	32	66	4	23	17	7	5	0	3	2
Cottontails (*Sylvilagus floridanus* subsp.)															
5–24–46	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0
1–27–47	10	7	2	1	3	42.8	1	0	0	0	0	1	0	0	0
3–3 47	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0
4–20–47	10	8	2	0	2	20	0	0	0	0	0	0	0	0	0
Total	24	19	4	1	5	20.8	1	0	0	0	0	1	0	0	0

bored 3 mature flukes and 3 had only eggs in the gall bladder. The lower incidence of fluke infection in the cottontails may be due to their inhabiting the higher areas, particularly along hedge rows and under remote ranch buildings such as barns and feed store-houses (Table 1).

The flukes were found, for the most part, in the common bile duct. Their presence caused extreme hypertrophy of the duct walls, resulting in extensive malformation.

DISCUSSION

Wild rabbits and hares in regions where liver flukes are present serve as a vast reservoir of infection. The finding by Pillers (1926) that wild rabbits in an area where sheep had not grazed for over 7 years were heavily infected with liver flukes, and the report by Montgomerie (1931) that liver flukes live for 37 months in domestic rabbits point to the potentialities of these animals in perpetuating infections in areas where the snail intermediate host is present. Moreover, in our studies, fluke eggs taken from the gall bladders of both cottontails and jack rabbits were fertile, and hatched when incubated at room temperature in the laboratory.

In addition to maintaining an infestation on unpastured areas, rabbits serve to intensify it when grazing with cattle and sheep. In the Gulf Coast prairie, where rabbits and the snail intermediate host of the liver fluke are numerous, the conditions for maintaining intense infestation are abundantly supplied. On ranches where careful range management is practiced and pastures are stocked to capacity, the rabbit population frequently far exceeds that of the cattle. Both the rabbits and the cattle seek the choice grazing areas. In these places there is an intense distribution of rabbit pellets containing large numbers of fluke eggs. The widespread distribution of the egg-filled rabbit pellets affords great opportunity for infection of the snails, and subsequently heavy infestation of the pasture with the infective cysts of the fluke. For these reasons, control of liver flukes in cattle and sheep by medication is rendered less effective where rabbits maintain a high output of viable fluke eggs.

SUMMARY

1. Wild rabbits and hares serve as natural hosts to the common liver fluke, *Fasciola hepatica*.

2. In the Gulf Coast region of Texas, 99 (32 per cent) of 309 jack rabbits, and 5 (20.8 per cent) of 24 cottontails were found to be infected with liver flukes.

3. Of the 99 infected jack rabbits, flukes were found in the livers of 56 (56.6 per cent), whereas only eggs of the flukes were found in the gall bladders of 43 (43.6 per cent).

4. In the 24 cottontails, flukes were found in 1 and only eggs in 4.

5. The number of flukes found in the jack rabbits ranged from 1 to 7 per animal; 3 flukes were found in the cottontail.

6. The flukes were located, for the most part, in the common bile duct, which showed extensive hypertrophy and malformation.

7. Eggs collected from the gall bladders hatched following incubation at room temperature.

8. The incidence of infection remained high throughout the year, as shown by frequent collections at various seasons.

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MAMMALIAN BLOOD FLUKES OF CELEBES

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INTRODUCTION

Previous to 1936 schistosomiasis japonica was known to be indigenous in Japan, China, Formosa, and the Philippine Islands. On April 1, 1936, a 35-year-old native tribesman from the village of Tomado, on the west shore of the small inland Lake Lindoë, Celebes, appeared in the provincial hospital of the near-by coastal town of Paloe, for treatment of a deep contusion of the neck. Physical examination revealed that, in addition to the injury, the patient was seriously ill; he was dyspneic and jaundiced. There was evidence of marked thoracic edema. Percussion and auscultation demonstrated fluid in the pleural cavity and rhonchi in both lungs, while there was also evidence of ascites, a nodular liver and a spleen enlarged three fingers below the costal margin. During the night death occurred suddenly and on the following day a partial autopsy was performed. There were bilateral fibrinous pleuritis, an enlarged cirrhotic liver and an enlarged spleen. Although a presumptive diagnosis of malaria was entertained because of its hyperendemicity in the Lake Lindoë villages, blocks of liver and spleen were fixed in formalin for later study in Java. Microscopic examination of the liver revealed helminth eggs which were at first thought to be those of *Paragonimus* (Müller and Tesch, 1936). Soon thereafter Professor S. L. Brug and Doctor Tesch conducted a survey of the Lake Lindoë area and demonstrated the endemicity of *Schistosoma japonicum* (8 per cent) and *Echinostoma* sp. (47 per cent) in the inhabitants of the three villages on the west shore of the lake (Brug and Tesch, 1937). In the same publication Müller and Tesch (1937) reported their autopsy in some detail and, following the demonstration by Professor Brug that the eggs in the section were those of *Schistosoma japonicum,* corrected their erroneous diagnosis of the previous year.

In 1939 Bonne, accompanied by Doctor J. H. Sandground, made a parasitological reconnaissance of the Lake Lindoë region and eludicated the life cycle of the human echinostome, *E. lindoense,* which they described as a new species, (Sandground and Bonne, 1940). During the summers of 1940 and 1941 Bonne and a group of his students revisited the area and found that approximately fifty per cent of the 176 inhabitants of the three villages (66 in Langko, 59 in Tomado and 51 in Antja) were passing eggs of *S. japonicum* in their stools. These workers obtained schistosomes from one human autopsy, from dogs in each of the three villages and from two wild deer shot near the lake shore. No other domestic or wild mammals were seen in the area except wild carabao. Several species of snails were collected and sent to Professor J. C. Bequaert of Harvard University for identification but no Oncomelanias were in the collection.

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During the 1941 expedition a visit was also made to Lake Poso, some 100 kilometers to the southeast of Lake Lindoë. In this latter area no human or animal infections with schistosomes and no Oncomelanias were discovered. However, a number of fork-tailed cercariae, obtained from a single specimen of *Lymnaea* sp., were used to inoculate experimental rats and mice. Eight weeks later these animals were sacrificed. The rats were negative but the mice contained many minute adolescent schistosomes in their mesenteric veins.

A preliminary report of the 1940 and 1941 expeditions has been published (Bonne et al, 1942). The purpose of the present communication is to amplify the earlier data with more detailed morphological evidence. This study has been made by the senior co-worker on material sent to him by Professor Bonne in 1941 and 1947 as well as on specimens from the same collection generously lent by Dr. J. H. Sandground.

GEOGRAPHICAL AND ANTHROPOLOGICAL DATA

Lake Lindoë is a small inter-mountain body of water in the northern part of Central Celebes, measuring about 10 kilometers in length by 5 to 6 kilometers in breadth (Fig. 1). It has an elevation of 960 meters above sea level. There are several small streams flowing into the lake on its southern and eastern shores, while its outlet is to the north, into a branch of the Paloe river which flows north-northwest to empty into Makassar Strait. The lake is surrounded by mountains which for the most part descend to the lake shore, although at certain locations there are stretches of flat sandy marsh, with an abundance of gastropods and bivalves. The lake is deep and abounds in fish, while large numbers of water birds congregate on its shores. The three villages of the immediate area, Langko, Tomoda and Antja, are all situated near one another on the west shore, and are reached by a single foot path from the southwest.

Like all the other peoples of Central Celebes the natives of the Lake Lindoë villages belong to the Toradja tribe, but they are particularly primitive because they are far from the main route of travel (30 kilometers distant) and have very little communication with other villages. Hookworm infection is prevalent (40 per cent) in all three villages, ascariasis was found in 9 out of 66 inhabitants at Langko (the southernmost village) and in 6 out of 59 at Tomado (the middle village). The inhabitants are apparently free of trichocephaliasis (Brug and Tesch, 1937). *Echinostoma lindoense* is a common parasite due to the native custom of eating raw molluscs (*Corbicula, Viviparus*) harboring the encysted metacercariae of this parasite (Sandground and Bonne, 1940). As indicated previously, schistosomiasis is prevalent in all three villages, and malaria is hyperendemic.

Lake Poso is much larger than Lake Lindoë, measuring about 40 kilometers in length by 20 kilometers in breadth. Its elevation is 510 meters above sea level. Its outflow is northward into a river which empties into the Gulf of Tomini. Lake Poso lies 100 kilometers distant from Lake Lindoë and is separated from the former lake by mountains covered with rain forest. There are several fairly large thriving villages around Lake Poso. The inhabitants have ready access by road to the sea on the north and thus are not isolated.

DESCRIPTION OF THE BLOOD FLUKES

Schistosoma japonicum.—The clinical picture of advanced schistosomiasis japonica in the villages of Lake Lindoë, with manifestations of splenomegaly, hepatic

cirrhosis, ascites and emaciation, duplicates that which the senior co-worker observed some years ago in China and Japan (Faust and Meleney, 1924) and more recently in the Philippines (Faust, Wright, McMullen, and Hunter, 1946). Moreover, in their several diagnostic characters the mature worms examined from human, canine, and cervine hosts are typical of *Schistosoma japonicum*. Several pairs as well as

FIG. 1. Lake Lindoë and environs, Celebes. The three black dots indicate the approximate locations of the three villages, Langko (southernmost), Tomado (middle) and Antja (northernmost). Adapted from "Sketch Map of Middle Celebes" by Dr. A. C. Kruijt, kindly provided by Colonel Tom Whayne, M.C., U.S.A., Preventive Medicine Service, Office of the Surgeon General.

separate males and females were found in the formalin-preserved specimens examined (Figs. 2–6).

The mature males (Fig. 2) measure from 18 to 21 mm. in length. The more delicate cylindrical female (Figs. 2, 3) has a length of 12 to 16 mm. In the male the testes (Fig. 2) are characteristically irregularly ovoidal, are compressed side-by-side

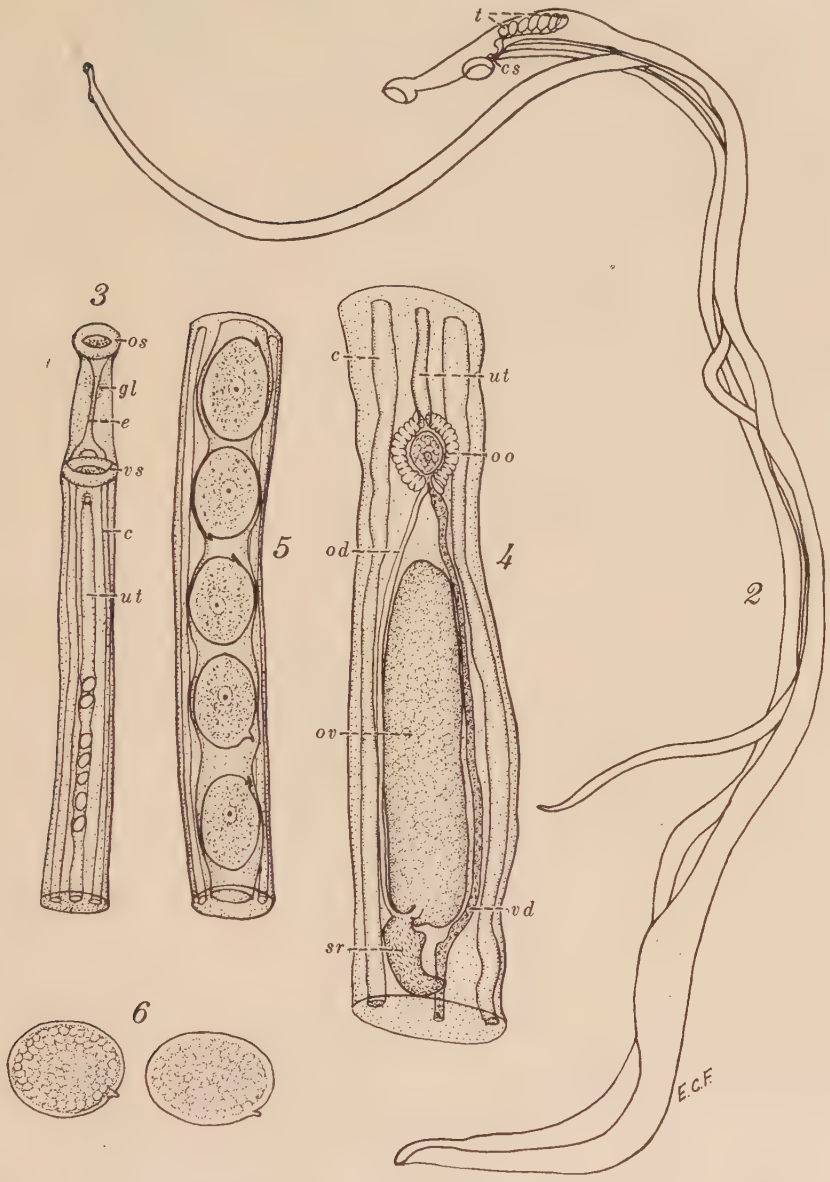

FIG. 2. Paired mature *Schistosoma japonicum* from mesenteric vein of naturally infected deer, Lake Lindoë. The female is held in the gynecophoral canal of the male. Seven testes (*t*) and the cirrus sac (*cs*) are seen just posterior to the ventral sucker in the male. ×12.

FIG. 3. Anterior end of mature female from mesenteric vein of deer, showing oral sucker (*os*), esophagus (*e*) surrounded by a cluster of glands (*gl*), ventral sucker (*vs*), cecum (*c*), and in the mid-line the outer portion of the uterus (*ut*) with several eggs. ×38.

FIG. 4. Portion of same worm as in Fig. 3 in vicinity of the ovary, showing cecum (*c*), ovary (*ov*), common vitelline duct (*vd*), seminal receptacle (*sr*), oviduct (*od*), oötype with Mehlis' glands (*oo*), and inner end of uterus (*ut*). ×200.

FIG. 5. Segment of same worm showing immature eggs, with characteristic, sharp-pointed spine, in inner portion of the uterus. ×200.

FIG. 6. Eggs from outer portion of the uterus. They are partially embryonated and the spine is blunter. ×200.

to form an elongated ovoidal mass and number 6 to 7 (6 in four and 7 in one of the specimens from man, consistently 6 in three specimens from the dog, and 6 in two and 7 in one of the specimens from the deer).

In the female the elongated ovary (*ov*, Fig. 4) lies medially a short distance behind the equatorial plane. Immediately posterior to its distal margin there is on the right side a distinctly incurved, swollen seminal receptacle (*sr*), which discharges spermatozoa into the oviduct (*od*) as this tubule bends out of the posterior end of the ovary and begins its ascent lateral to the ovary en route to the oötype. The paired vitelline ducts unite to form the common vitelline duct some little distance behind the level of the seminal receptacle. This tubule (*vd*) proceeds anteriorward on the left side of the ovary and enters the oötype at the side of the oviduct. The oötype (*oo*) is provided with a thick glandular inner wall, comprising Mehlis' glands. The uterus (*ut*) arises from the anterior aspect of the oötype and then ascends its long course (approximately 10 mm) to the genital pore which lies medially just behind the ventral sucker.

The uterus in the Celebes material has a capacity of approximately 200 eggs; from 60 to 100 were counted in mature specimens. The transverse diameter of the partially embryonated eggs is somewhat greater than the normal diameter of the mid-uterus (Fig. 5), so that each egg causes a distention of the uterine wall, with a slight intermediate constriction. By the time the eggs are pushed forward into the outer portion of the uterus they have increased in size and embryonation is about half completed (Fig. 6). The shell is still biconvex, and does not assume its rotund contours until after oviposition. A consistent characteristic of the uterine eggs in all specimens of the Celebes material is the conspicuous, incurved, lateral spine. In the inner portion of the uterus (Fig. 5) it is sharply pointed but nearer the genital pore it is blunter (Fig. 6). In experimental and natural *S. japonicum* infections in China the presence of this spine was usual (Faust and Meleney, 1924) but in Philippine material there were many incompletely embryonated eggs in which the spine was not observed or was relatively inconspicuous (Faust, 1946).

The large number of uterine eggs in *S. japonicum* from the Celebes material, as in that studied by several workers in Japan, China, and the Philippines, suggests relatively great fecundity of the females of this species of blood fluke. The contrast between egg production in *S. japonicum* and *S. mansoni*, as previously noted (Faust and Hoffman, 1934), provides a rough measure of the relative tissue damage produced by these two schistosomes, since intestinal fibrosis, hepatic cirrhosis and ectopic granulomas in these infections are due primarily to the infiltration of eggs into the tissues, with the formation of a pseudo-tubercle around each egg.

The Lake Poso Blood Fluke.—Only a few mature cercariae and several immature worms have been available for study (Figs. 7–10). The formalin-fixed cercariae are relatively robust and have the following measurements: body length, 0.15 mm, breadth, 0.080 mm, anterior (preoral) sucker, 0.045 mm, ventral sucker, 0.023 mm, tail trunk, 0.225 mm, furcae 0.10 mm. The body and tail are covered with minute spines diminishing in size and number from anterior to posterior end. There are no eye spots and there is no evidence of a pharynx. Lying medially on the dorsal side of the preoral sucker there is a conspicuous, thick walled head gland (*hgl*). Just underneath the posterior margin of this sucker there is a minute oral opening (*e*), with a capillary esophageal tube leading inwards. The most conspicuous

feature of the body of the cercaria is the penetration-gland complex (*pgl*). As in most schistosome cercariae, so in this one the paired unicellular glands lie in the posterior half of the body, lateral and dorsal to the ventral sucker, and their ducts open through spinose tips dorsolaterally to the opening of the anterior sucker. Only three pairs of these glands have been found in the Lake Poso cercaria.

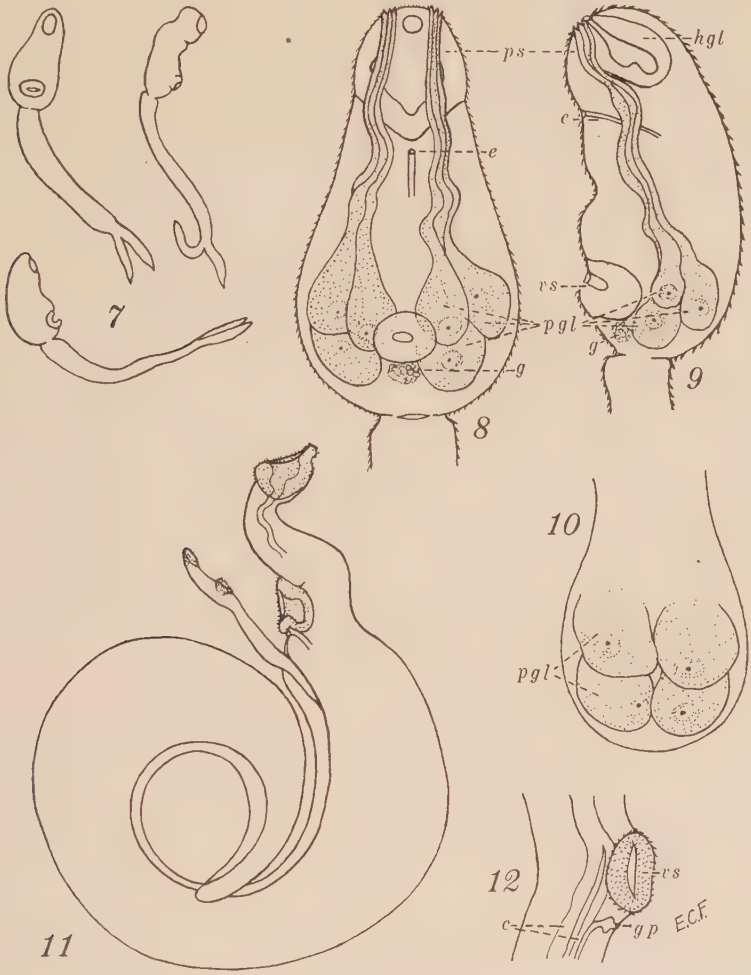

Fig. 7. Three camera lucida sketches of cercariae from *Lymnaea* sp. at Lake Poso. ×83.

Figs. 8–10. Views of the body of the cercaria, showing preoral (*ps*) and ventral (*vs*) suckers, head gland (*hgl*), oral opening and esophagus (*e*), penetration glands (*pgl*) and genital primordium (*g*). 8, ventral view; 9, lateral view; 10, dorsal view showing the two pairs of penetration glands seen from this aspect. ×280.

Fig. 11. Paired imature male and female schistosomes from mesenteric vein of mouse exposed to furcocercous cercariae obtained from *Lymnaea* sp. at Lake Poso. Note resemblance to species of *Schistosoma*. ×95.

Fig. 12. Detail of region of ventral sucker of immature male schistosome from mouse experimentally infected with Lake Poso cercariae. Lateral view. *vs*, ventral sucker; *c*, intestinal ceca; *gp*, genital pore. ×350.

The worms obtained from the mesenteric venules of the mouse exposed to the cercaria are very small and immature (Fig. 11, 12). The males measure approximately 2.2 mm in length by 0.18 mm in cross section and the females, about 1.6 mm

and 0.057 mm, respectively. Some of the worms were mated, others were separate. The suckers of both sexes are delicately spinose but otherwise the cuticula is glabrous. A genital atrium and pore (*gp*) have been found in the male (Fig. 12) but the testes have not been demonstrated. No internal structures of the females have been observed.

While it is possible that the worms may be dwarfed because they developed in an inappropriate host, or that they were immature because they were not given adequate time to complete development, it seems equally possible that this species of blood fluke is small compared with known representatives of the genus *Schistosoma.*

The generic and specific identification of the Lake Poso blood fluke can not be made on the basis of the material available for study by the senior co-worker. The molluscan host (*Lymnaea* sp.) and the number of penetration glands of the cercaria are suggestive of *Schistosomatium* but eye spots are lacking. Furthermore the adolescent worms are much more cylindrical and stouter than the described species of *Schistosomatium* (i.e., *S. pathlocopticum* Tanabe, 1923 and *S. douthitti* [Cort, 1913] Price, 1931). On the other hand, both cercariae and adolescent worms resemble species of *Schistosoma,* although the smallest number of penetration glands described for cercariae of this genus is eight (*S. bovis*) and the more usual number is ten (*S. japonicum, S. haematobium*). It is possible that the Lake Poso blood fluke is an undescribed species of *Schistosoma.* Moreover, Sewell (1919) and Porter (1938) have regarded cercariae obtained from *Lymnaea* spp., similar to that from Lake Poso except for the possession of five pairs of penetration glands, as the larval stage of *S. japonicum.* The evidence in this case (molluscan host, structure of the cercariae, stout, cylindrical appearance of the adolescent worms and failure to find the infection naturally in man and other mammalian hosts in the area) practically exclude the possibility that it is *S. japonicum.*

SUMMARY

1. Circumstances are recounted of the discovery of schistosomiasis japonica among primitive natives of the three villages on the west shore of Lake Lindoë, an isolated region of Central Celebes. Mature worms obtained from autopsy of man, dogs and native deer, studied with special reference to their morphological characteristics, confirm the pathological and survey diagnoses, that *Schistosoma japonicum* is autochthonous in this area.

2. Study of furcocercous cercariae obtained from a *Lymnaea* sp. at Lake Poso, Celebes, and of the immature worms obtained from mice after exposing them to these cercariae demonstrate that they belong to the family SCHISTOSOMATIDAE, with certain cercarial characteristics resembling those of *Schistosomatium* but lacking eye spots. This cercariae more closely approximates this stage of species of *Schistosoma* in its general appearance and type of penetration glands, but the number of these glands (three pairs) is fewer than that described thus far for the cercaria of any species of this genus. Moreover, the immature worms are more like *Schistosoma* than *Schistosomatium,* but are not generically diagnosable because of their immaturity. However, it is concluded that this schistosome is not *Schistosoma japonicum.*

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AN APPARATUS FOR REMOVING TROPICAL RAT MITES FROM LARGE QUANTITIES OF BEDDING MATERIALS[1]

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For certain experiments involving the use of the tropical rat mite, *Liponyssus bacoti,* as vector of the cotton rat filaria, *Litomosoides carinii,* it has been necessery to make rapidly available several thousand adult mites from a single source. A method for producing the required number of mites by raising them on a white rat or a cotton rat in a metal tank containing wood shavings and hay has already been described (Scott, Stembridge, and Sisley, 1947). The apparatus shown in Fig. 1 has been constructed for the purpose of extracting within a single working day this large number of adult mites and the even larger number of accompanying nymphs from approximately 4000 cc of wood shavings and an equal volume of hay.

The principal on which the apparatus is based is that used by Bertram (1946) in an apparatus for removing mites from small quantities of sawdust, namely that of driving the mites ahead of a wave of heat. Because of the problem of condensation of moisture the closed system of Bertram was replaced by an open trough of galvanized sheet iron surrounded by a moat containing water. Instead of a hot wire heating element there was substituted an infra red bulb of 250 watts, the back of which is reflector coated. This bulb is mounted on a carriage containing two pulleys which run on a wood track at a sufficient height above the trough to cause the principal heating area to slightly more than cover the width of the trough. The motive power is provided by a small electric motor turning a glass tube. A heavy cotton cord from the lamp carriage is wound twice around this tube, then passed through a pulley and attached to a weight. The length of the cord is adjusted so that the weight will rest on the floor just before the lamp reaches the end of the tank, relieving the tension and allowing the cord to slip. Thus if the operator is absent there will be no damage to the mechanism and any mites remaining at the end of the trough will not be killed by excessive application of heat.

The lamp moves at the rate of $\frac{1}{2}$ inch per hour and will heat a 1 inch layer of sawdust or a 4-inch layer of loose hay. It was found that by the time mites stranded on the end of a piece of hay were forced to drop off, the metal bottom was so hot that they were killed. The bottom of the trough was therefore lined with a $\frac{1}{4}$-inch sheet of hard asbestos-cement composition board to delay conduction of heat along the bottom. An auxiliary hot wire heater can be hung from the lamp carriage beneath the trough and just behind the center of the lamp so as to heat a thicker layer of sawdust from beneath.

When the mites are not too numerous they can be removed from the inner top edge of the trough by means of a suction bottle. One hole of the rubber stopper in this bottle is covered with bolting silk and attached by rubber and glass tubes to a suction pump, while the other hole is provided with a tapered and bent glass tube into which the mites are sucked. Some mites will fall into the water and can be

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[1] The work on which this paper is based was supported by a grant for the study of filariasis from the John and Mary R. Markle Foundation.

removed with a camel's hair brush to a piece of filter paper where they soon dry and can be picked up in the bottle. When the number of mites is large they can not be removed from the water rapidly enough to keep up with the movement of the lamp. Consequently a copper tube of $\frac{1}{8}$-inch bore is soldered into a hole in the center of the bottom of the moat at the narrow end of the trough to form an overflow drain, thus maintaining the water at $\frac{1}{4}$-inch depth. At the other end an intermittent siphon discharges about 20 cc of water every two or three minutes so as to flush any mites in the water into the overflow tube. The frequency of discharge of the siphon must be carefully adjusted so that the flow will be sufficient to prevent large numbers of mites accumulating in the water, but not sufficient to appreciably cool the sides of the trough at the point where the light is shining on it. The overflow tube is con-

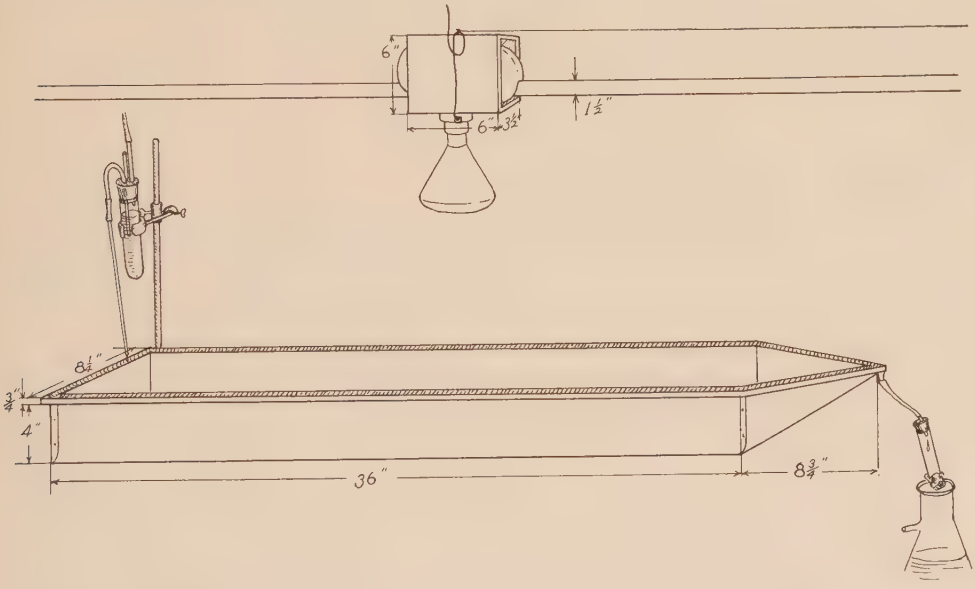

FIG. 1. An apparatus for driving tropical rat mites from bedding materials by means of heat from a moving infra red lamp.

nected with rubber and glass tubes to a glass tube 10 cm long and 2 cm in diameter, the bottom of which is covered with No. 12 XX bolting silk. Whenever this silk becomes covered with mites the tube is replaced by another. The full one is then attached to a suction pump and air from a bottle containing a desiccant is drawn through it until all visible moisture is removed. The rubber stopper is replaced with a solid one and the tube is kept in a refrigerator at about 5° C until the mites are needed.

The adult mites are separated from the nymphs by inverting the unstoppered tube on a piece of white paper under a strong light. As the mites crawl out from beneath the tube the two stages are picked up separately in two suction bottles.

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DESCRIPTION OF THE MALE OF *ANDROLAELAPS SETOSUS* FOX (ACARINA: LAELAPTIDAE)[1]

D. C. Thurman, Jr.,[2] Nina Branch,[3] and John A. Mulrennan[4]

Fox (1946) reported the occurrence of three new species of mites from rats in Puerto Rico, among them a laelaptid which he described from the female holotype as *Androlaelaps setosus*. Seventy-eight females and three males of this species have been combed from rats in widely separated areas in Florida during the course of evaluation studies of the control of rat ectoparasites with DDT.

Family Laelaptidae

Androlaelaps setosus Fox

Description of the male allotype (Fig. 1). Length 0.55 mm. Width 0.35 mm. Smaller than the female. Body oval in shape, longer than broad.

The five segmented palpus shows a heavy, curved seta on the outer side at the distal end of the penultimate segment. The epistome is well supplied with many fine spicules.

As shown in Fig. 2, the fixed chela of each chelicera is reduced and bears a single stout seta; the movable arm is strong, stout, about four times as long as its basal width, slightly curved near the anterior end and with a semicircle of close, fine setae at its base. From this base a leaf-like process arises and extends about three-quarters of the length of the arm.

The ventral plates are united, distinct, with a network of numerous fine lines and ten pairs of long setae in addition to the three anal setae, arranged as shown in Fig. 1. Of the paired setae, three pairs belong to the sternal region. The genital pore and one pair of these setae lie at the anterior margin and two pairs near the lateral margins of the plate. Of the lateral pairs, one is at the level of the posterior margin of coxa II and the other at the level of the anterior margin of coxa III. Seven pairs of setae belong to the genito-ventral region. Of these, one pair lies at the level of the posterior margin of coxa III, one pair at the level of the posterior margin of coxa IV, with the other five pairs scattered over the plate posterior to the fourth coxa. The paired anal setae are located laterad of the anal pore and slightly forward of the posterior margin of the pore. The final unpaired seta lies caudad of the anal pore. Along the margin of the holoventral plate on either side below coxa IV, are twelve large, slightly and shortly branched setae curving inward toward the plate, while along the margins of the body there are six large, sparsely and minutely branched paired setae. The holoventral plate is widest behind coxa IV, where it extends laterad of the outer margins of the coxae.

A large, distinct genital pore is located at the anterior margin of the holoventral plate. On either side of the genital pore, a transverse slit-like pore is located just posterior of each seta of the first pair. Between the second and third pairs of setae, on both sides of the plate, there is an oblique slit-like pore, and between the third and fourth pairs of setae, a curved longitudinal pore. The anal pore (Fig. 3) is longer than broad and approximately the same size as the genital pore.

The peritreme extends anteriorly well beyond the first coxa. The stigmal pore is located laterad of the anterior margin of coxa IV, with several longitudinally arranged setae between it and the lateral margin of the body. The dorsal shield is entire, large, almost covering the body, with about twenty pairs of strong, sparsely and minutely branched, curved setae. On the dorsal posterior portion of the body, the setae are larger than those located anteriorly.

The legs are well provided with strong setae. Leg II, broad, with a stout thumb-like spur on the femur, the patella and tibia each with a short stout spur, the tarsus with two slender spurs and several pairs of heavy setae.

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[3] Medical Technologist, Division of Entomology, Florida State Board of Health.

[4] Director, Division of Entomology, and CDC Activities, Florida State Board of Health.

Type Material.—The androtype from *Rattus norvegicus* at Tampa, April 12, 1946, has been submitted to the specimen collection of the U. S. National Museum, Washington, D. C. Male paratype from *Rattus norvegicus* at Miami, January 24, 1947 in the collection of the Florida State Board of Health, Jacksonville, Florida. Female metatypes compared by Dr. Irving Fox with his type material in the entomological collections of the Department of Medical Zoology, School of Tropical Medicine, San Juan, Puerto Rico are in Dr. Fox's collection and in the collection of the Florida State Board of Health.

Acknowledgment is made of the assistance of Dr. Harry D. Pratt, Dr. R. W. Strandtman and Mr. G. H. Bradley who reviewed the description and whose suggestions were incorporated into the final illustration.

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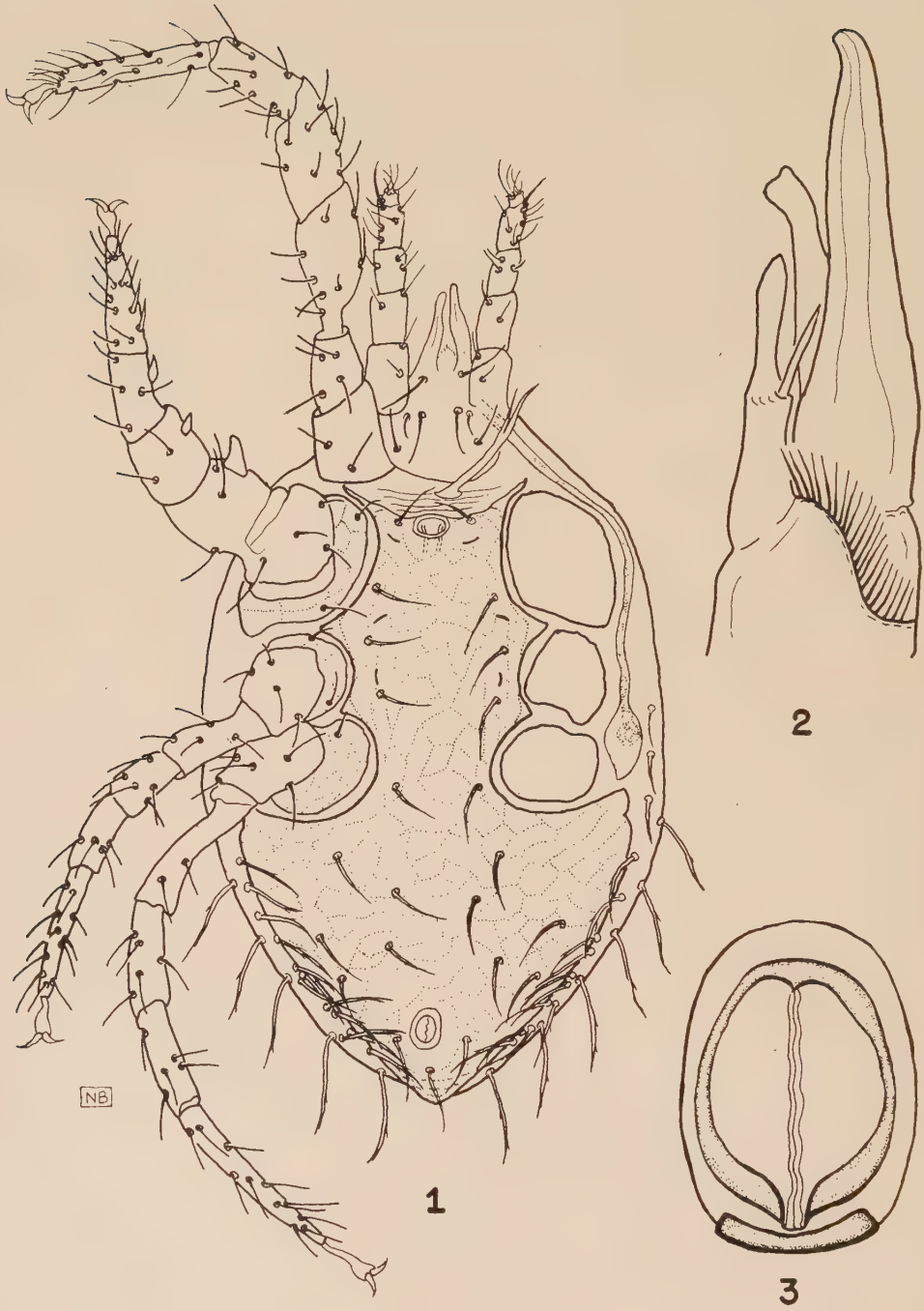

EXPLANATION OF PLATE

Male of *Androlaelaps setosus* Fox

FIG. 1. Ventral view of body.

FIG. 2. Chelicera, lateral view.

FIG. 3. Anal pore.

STUDIES ON EXPERIMENTAL BLACKHEAD INFECTION IN TURKEYS

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The difficulty with which *Histomonas meleagridis,* the causative agent of blackhead or enterohepatitis in turkeys, is consistently transmitted experimentally has offered a serious handicap to investigators undertaking chemotherapeutic studies of this disease. Many different methods of transmission have been used with some degree of success including the exposure of susceptible turkeys to premises in which chickens or turkeys had been or were being kept (Tyzzer, 1920a et seq), the feeding of soil from such premises (Tyzzer and Fabyan, 1922; Tyzzer, 1934), the oral administration of cultured organisms (Tyzzer, 1934) or diseased liver and cecal tissues (Tyzzer and Fabyan, 1922 et seq), the rectal administration of diseased tissues (Tyzzer and Collier, 1925) or cultured organisms (Delaplane, 1932), the intravenous inoculation of macerated diseased tissues (Tyzzer, Fabyan, and Foot, 1920), the subcutaneous implants with diseased tissues (Tyzzer and Fabyan, 1920), the intrasinal inoculation with diseased liver tissue (DeVolt and Davis, 1936), and the feeding of embryonated *Heterakis gallinae* eggs to susceptible turkeys (Graybill and Smith, 1920; Tyzzer and Fabyan, 1920 et seq).

Tyzzer (1920b), who found difficulty in the experimental production of the disease by exposure of normal birds to infected birds, also stated that repeated feeding of diseased tissues and discharges from cases of blackhead, failed to produce the disease in normal turkeys. Tyzzer and Fabyan (1922) again recorded "The feeding of blackhead lesions to normal turkeys has frequently resulted negatively." Tyzzer, Fabyan, and Foot (1920) found that intravenous inoculation of diseased tissues produced disease in only a small proportion of cases which was "in striking contrast to subcutaneous inoculation, which practically always causes infection."

Subcutaneous implants and intrasinal inoculation, while quite reliable as a means of transmission, produce atypical lesions and are therefore undesirable for routine laboratory purposes. On the other hand, the feeding of mature *Heterakis* eggs and rectal inoculation with cultured organisms or diseased tissues have produced typical lesions in a large proportion of the birds inoculated.

While other investigators have amply shown the effectivenes of mature *Heterakis* eggs as an infecting medium, the following data will emphasize the convenience, reliability, and practicability of this method as a routine laboratory procedure.

MATERIALS AND METHODS

All of the turkey poults used for testing were purchased when a day old from hatcheries and upon arrival at the laboratory, were immediately placed in brooder batteries. Special precautions were taken to protect the birds from extraneous infection. At the time the tests were started, the poults were taken from the batteries and

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placed in individual, wire bottomed, test cages, which had been thoroughly cleaned and disinfected.

Since preliminary trials using embryonated eggs of the cecal worm, *Heterakis gallinae,* as an infective medium were successful in our laboratory, we have adopted this method as a routine procedure. Ceca from adult chickens were obtained from commercial poultry dressing plants. The contents of the ceca were removed and the cecal worms separated from the excreta. The worms were then macerated in a mortar and incubated in 1.5 per cent formalin for about ten days at either room temperature or in an incubator at about 30° C. When the embryonation of the eggs was completed, they were separated from the formalin by centrifugation, placed in tap water, refrigerated and used as needed. The turkeys were infected *per os* once daily for three consecutive days, each bird receiving 175–875 embryonated eggs per day. We have been able to produce 80 per cent mortality in 313 infected control birds from 54 experiments by using this technique.

The syndrome characterized by sulfur-yellow colored droppings, inappetance, drowsiness, drooping wings, unthriftiness and general debilitation, was considered as evidence of blackhead. Reliability of these symptoms in our experimental birds, has been established by the fact that postmortem examination invariably showed lesions of blackhead in the liver and ceca resembling those described by Tyzzer (1920).

The unselected data presented herein, is from observations on the unmedicated, infected, control birds from a series of 54 experiments, conducted over a period of several years to determine the chemotherapeutic activity of various substances for controlling this disease.

EXPERIMENTAL

Fifty-four tests have been conducted utilizing 313 unmedicated, infected, control birds. Of these, 251 or 80 per cent of the birds died from blackhead. Table 1 shows the percentage mortality of the various experiments and the number of experiments falling within each mortality level.

Our data show that the major portion of the infected birds developed clinical symptoms in 12 to 14 days after cecal worm eggs were administered to the birds. We have not been able to produce clinical symptoms of the disease sooner than ten

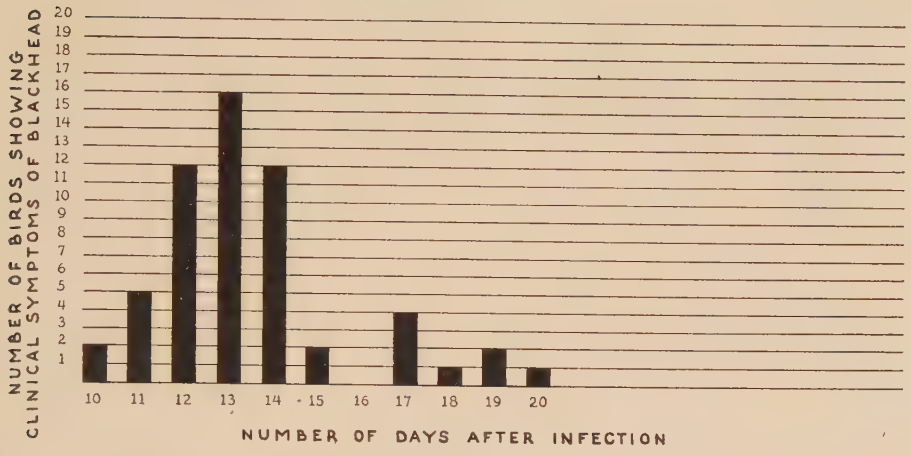
TABLE 1

Number of tests conducted	Number of birds utilized	Number of birds dying	Percentage mortality
1	4	0	0.0
2	8	2	25.0
5	24	12	50.0
1	20	12	60.0
1	28	18	64.0
1	3	2	66.6
12	56	42	75.0
1	5	4	80.0
2	12	10	83.3
3	24	21	87.5
1	16	15	93.8
24	113	113	100.0
Total 54	313	251	....

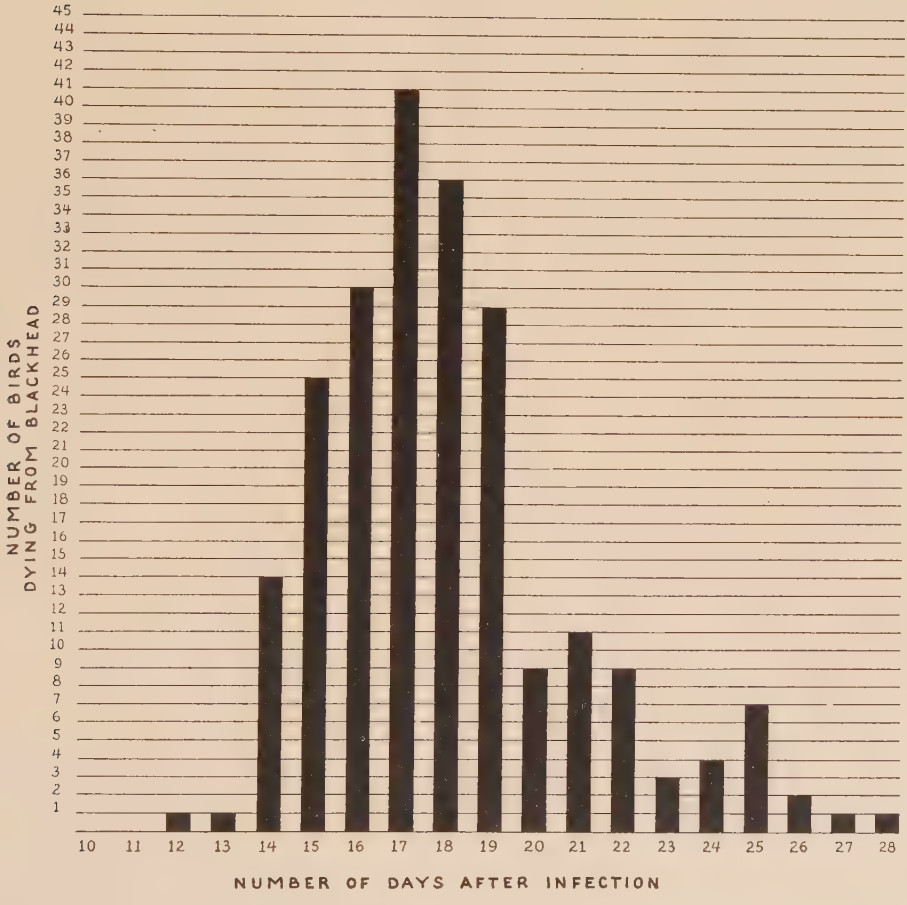
80.0 per cent mortality for 313 birds in 54 tests.

days after the primary infection (see Graph I). Mortality was greatest between 15 and 20 days after infection, reaching the peak at seventeen days (see Graph II). These observations are in agreement with those of Tyzzer and Fabyan (1920).

GRAPH - 1



GRAPH - 2



DISCUSSION

The ideal method of experimentally infecting turkeys with blackhead would be to administer pure test tube cultures of *Histomonas meleagridis*. However, in our laboratory only a relatively small percentage of attempted oral infections with such cultures have been successful. DeVolt and Davis (1936) found that 29 out of 33 poults inoculated orally and rectally with protozoan pure cultures, died from blackhead.

Another method of infection used by us was a modification of rectal injections used by Tyzzer and Collier (1925), Delaplane (1932), and Tyzzer (1934). The equipment consisted of a six-inch length of catheter fitted over the delivery tip of a 10-cc glass syringe. The required amount (2–5 cc) of test-tube culture, cecal droppings, cecal or liver tissue suspended in sterile saline, was drawn into the syringe and the catheter inserted into the cloaca until the distal end was in the approximate vicinity of the cecal opening. While the attendant held the poult, the operator, holding the syringe in the right hand, pushed the vent firmly around the catheter with the left hand and forced the infective material into the digestive tract. This method required little time and resulted in about 70 per cent successful infections when infective liver or cecal tissue was used. Disadvantages of these techniques are, (1) that the production of test tube culture is time consuming and the results uncertain; (2) the use of the infected liver or cecal tissues involves the risk of multiple infections with other protozoa or bacterial organisms; and (3) infective material is not always available when needed.

A more useful method of infection involves the use of embryonated eggs of the cecal worm, *Heterakis gallinae,* as previously reported by Graybill and Smith (1920), Tyzzer and Fabyan (1920), Tyzzer (1934 et seq). While Tyzzer points out that not all *Heterakis* ova carry blackhead infection, the writers have never failed to obtain infective material when a pooled culture of ova from a number of adult market fowl was used. Since the cecal worm eggs for our cultures have been collected from widely distributed areas, we are led to believe that a high percentage of mature chickens are carriers of *Histomonas meleagridis*. As previously stated, we have been able to obtain 80 per cent infection in these unmedicated, infected birds, 100 per cent infection in such birds frequently being attained for individual experiments (see Table 1). The use of *Heterakis gallinae* eggs from mature chickens for transmission of blackhead disease to turkeys has the following distinct advantages over the other methods attempted: (1) a high percentage of birds can be infected with this method; (2) infective material is readily available; and (3) infective culture material can be prepared and kept available at all times since we have found that infective cecal worm egg cultures retain their histomonal infectivity for more than a year when kept in an ordinary household refrigerator.

Since a successful experimental method for transmission of blackhead is available, the writers contend that experimental infection should form the basis for therapeutic studies of this disease. Field trials, controlled or otherwise, should be used only to supplement data obtained from controlled tests, wherein the birds are individually infected by experimental means. The sporadic occurrence of blackhead in flocks kept on contaminated soil or in association with infected chickens or turkeys (Tyzzer, 1920; Tyzzer and Fabyan, 1920) makes data from such therapeutic studies unreliable, since the question always arises as to whether the treatment under

consideration actually controlled the disease or whether the treated birds merely failed to acquire the disease.

SUMMARY

1. Various methods of infecting turkeys experimentally with *Histomonas meleagridis,* the organism causing blackhead infection, are discussed.

2. Utilization of the embryonated eggs of the cecal worm, *Heterakis gallinae,* is shown to be a convenient and reliable method for producing blackhead in turkeys.

3. The unreliability of field trials depending on the sporadic occurrence of blackhead is discussed.

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EFFECT OF STREPTOMYCIN ON *ENDOLIMAX NANA IN VITRO**

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INTRODUCTION

Following the discovery of streptomycin by Schatz, Bugie and Waksman (1944), numerous attempts have been made to ascertain the effect of this antibiotic on pathogenic bacteria. There also have been some attempts to determine the effect of streptomycin on protozoa. Balamuth and Wieboldt (1946) noted that trophozoites of *Endamoeba histolytica* survived exposure to 10,000 units per ml for 48 hours although relatively few recovered. Anderson, Villela, Hansen, and Reed (1946) reported that streptomycin in 1 : 2,500 dilution killed *E. histolytica in vitro*. Spingarn and Edelman (1947) reported that addition of streptomycin (1000 to 3000 units per cc) prolonged the survival of cultures of *E. histolytica* from an average of 8.0 days to 33.7 days and eliminated the necessity of frequent transplants to maintain the strain. This effect was associated with evidence of bacterial inhibition but not with sterilization of the culture.

The present paper records the first attempt to determine the effect of streptomycin sulfate and its residue on cultures of *Endolimax nana.*

MATERIALS AND METHODS

A strain of *E. nana* (A_3), isolated from a human stool, was maintained on modified L.E.S. medium from February 10, 1947, until the end of May 1947, when the work was discontinued. The techniques employed were based on reports of Balamuth (1946), Dobell (1927, 1928, 1940, and 1942), Dobell and Laidlaw (1926), Fonda (1928), Felsenfeld and Young (1945), and texts of Craig (1934) and Mackie, Hunter and Worth (1946).

Numerous attempts were made to transfer this strain to other culture media. All failed to give as constant and heavy a growth as L.E.S. medium. Balamuth's medium was one of those which was tried several times, but the amoebae failed to grow on it. The best results were obtained with L.E.S. medium when Ralston whole wheat flour was substituted for rice.

Streptomycin sulfate, a highly purified crystalline salt, supplied by Charles Pfizer & Co., Inc., lot P-472, which had a potency of 818 mcg/mg was used. Streptomycin residue, also supplied by the same company, was employed. This material contained streptomycin B and a considerable amount of impurities, and had a potency of 165 mcg/mg. The dry streptomycin and stock dilutions of both streptomycin sulfate and its residue were kept in a refrigerator at 10° C. Fresh solutions were made up every two weeks. Streptomycin sulfate, made up in neutral distilled water, was introduced into culture tubes so that the total concentration was in the range of 20–16360 units per ml. The amoebae were left in contact with the streptomycin sulfate for 240–288 hours. Streptomycin residue was dissolved in neutral distilled water, and then filtered through a Jenkins filter. The total concentration of the

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* Contribution from the Biological Laboratory, New York University, University Heights, under the direction of H. W. Stunkard.

residue in the culture tubes was in the range of 27–825 units per ml. The amoebae were exposed to this substance for 120–384 hours.

Both stock cultures and experimental cultures were transferred every 48 hours. One ml of the culture, rich in amoebae, was transferred to each new test tube. In all cases, 24- or 48-hour cultures were inoculated with the substance used. Each culture was checked for growth of amoebae prior to the addition of streptomycin or its residue. Observations for growth were made every 24 hours for the first 72 hours, and subsequently every 48 hours. In all cases the general appearance, increase or decrease in number, and the motility of trophozoites were compared with stock cultures which were used as controls. The pH of each individual culture was determined prior to inoculation, every 24 hours for the first 3 days and then at 48-hour intervals. The Fonda Spot Method of pH Determination was used. No attempts were made to observe the amount of inhibition that streptomycin had on the bacterial flora of the cultures, and there was no plating out of bacterial strains.

A uniform sized drop was picked up from the bottom of the test tube, and examined for five minutes. Growth was considered "good" when the amoebae in experimental cultures were as numerous as in the controls. A "heavy" growth implies that the number of amoebae exceeded that in the controls. Growth was considered "poor" when the number of amoebae fell below the normal observed in stock cultures. Thus the number of amoebae per fields examined, was interpreted as "heavy," "good," or "poor."

In old cultures, the medium was turbid, and consequently it was difficult to control the exact amount of fluid to be taken up in a pipette. Accordingly examinations were made after the culture was transferred to fresh media, and allowed to stand in the incubator (37.2° C) for one hour. In this manner it was possible to obtain a uniform amount of the culture medium for examination, and the turbidity which was normally present in the old culture tube was absent in a fresh tube after the transfer.

DISCUSSION OF RESULTS

The results of the experiments are summed up in tables 1, 2, and 3. It becomes apparent from table 1 that the amoebae are capable of tolerating a wide range of pH. As a matter of fact, in several instances, growth was considerably better both in the controls and experimental cultures at pH 6.0–6.5, than in a more alkaline pH, pH 8.0–8.4. In the lower pH range indicated above, the amoebae grew better, their motility was increased, and there were fewer spherical, immobile, or degenerate forms. The fact that growth of amoebae was better when the pH was low (6.0–6.5) may be due to inhibitory effects of this hydrogen ion concentration range on the multiplicative rate of the bacteria in the culture. This favorable pH range may possibly be correlated with the beneficial effect of Ralston whole wheat flour since the pH became lower when wheat was used.

Rawson & Hitchcock (1947), working with *Endamoeba histolytica,* allowed 24 hours for contact with the drug that was tested, but it seemed more desirable in the present experiment to expose the amoebae for a longer period. The reason for this was twofold; firstly, because it was thought that there might be a cumulative action of the agent, and secondly, as suggested by Brackett & Bliznick (1947), there was the possibility that a large part of the agent might be absorbed by the egg-slope. However there seemed to be no accumulative action, and as for the second pos-

sibility there is no proof at present that it did not happen. Therefore it was thought that a longer period of contact with the antibiotic would compensate for the amount of the agent absorbed.

TABLE 1.—*Controls (stock cultures)*

Symbols:
A_3W = *E. nana* in L.E.S. medium with Ralston whole wheat flour added.
A_3 = *E. nana* in plain L.E.S. medium.

Culture	pH range		Growth	Period of observation
	Initial	Final		
1. A_3W ...	7.8–7.0	6.5–5.7	good → poor → good	43 days (1032 hours)
2. A_3	8.4–7.0	7.5–6.8	good → poor → good	43 days (1032 hours)

The results obtained here do not agree with those of either Balamuth & Wieboldt or Anderson et al, although it is recognized that they were working with a different intestinal amoeba.

TABLE 2.—*Effect of streptomycin sulfate*

Culture	Streptomycin sulfate; units per ml	pH range		Growth	Total time in contact with streptomycin
		Initial	Final		
					hours
1. A_3	16360	7.2–6.8	6.9–6.5	heavy	288
2. A_3W ...	8180	7.4–7.0	6.0–5.7	good	288
3. A_3W ...	818	7.7–7.4	6.5–5.6	good	240
4. A_3W ...	82	8.0–7.0	6.5–5.7	good → poor	240
5. A_3	41	8.2–7.4	7.1–6.8	poor → good	240
6. A_3	27	8.0–7.4	7.4–6.9	good	240
7. A_3	20	8.4–7.0	7.5–6.8	good → poor → good	288
8. A_3W ...	20	8.2–7.8	7.3–6.5	good → poor	288

In general, streptomycin sulfate and its residue (tables 2 and 3) in concentrations employed, affected the growth of *E. nana* beneficially, with no apparent detrimental effects on the amoebae. Indeed, in most cases, the organisms increased in number and in activity from the first 48 hours to the end of the period of exposure.

This effect may be an indirect one. As mentioned above, the strong bacteriostatic qualities of streptomycin may have reduced the numbers of those bacteria which are not essential to growth of the amoeba, since *E. nana* requires a certain amount of bacteria in the culture (Dobell, 1933).

The only case of a culture dying out was that of A_3 at 41 units per ml of streptomycin residue (table 3). This loss is not significant, because another culture was able to survive in the same concentration for a period of 120 hours, and improved from "poor" to "good" growth.

Two cultures of *E. nana,* both with whole wheat flour, one at 818 units, and the other at 20 units of streptomycin per ml, went from "good" to "poor." This reduction might be interpreted as an effect of streptomycin. However, it should be pointed out that normal fluctuations, which have been attributed to the bacterial flora, occur in cultures of amoebae. Fluctuations in growth of the amoebae in stock cultures may be observed from day to day. Other workers, including Thomson and Robertson (1925), have noted normal fluctuations in cultures of other intestinal amoebae. The fact that growth was good for the first 48 hours further

indicates that the decline in number of amoebae probably was not a direct effect of streptomycin. The appearance of the trophozoites in experimental cultures did not differ in any way from those of the controls which were examined at the same time. Since concentrations as high as 16360 units per ml seemed to augment the growth of the amoebae, the inhibitory effect of streptomycin in lower concentrations is improbable. Nevertheless the possibility, that streptomycin in lower concentration inhibits the bacterial growth to a lesser degree, and consequently, the effect on the survival of the amoebae is unfavorable, is recognized. This indirect effect of streptomycin, if it may be called such, is a negative one, and is certainly not as pronounced as the more favorable effect on the amoebae.

TABLE 3.—*Effect of streptomycin residue*

Culture	Streptomycin residue : units per ml	pH range		Growth	Total time in contact with streptomycin residue
		Initial	Final		
					hours
1. A_3W ...	825	8.2–7.5	7.0–6.5	good	384
2. A_3W ...	82.5	8.0–7.4	7.0–6.3	good	384
*3. A_3	41	8.0–7.3	7.5–7.3	good → died out	264
4. A_3	41	8.0–7.8	7.5–7.3	poor → good	120
5. A_3	27	8.0–7.2	7.3–6.8	poor → good	384

* The number of amoeba in culture No. 3 steadily declined and it subsequently died out. The causes were undetermined. Another culture No. 4 was reinoculated in the same concentration as a check on No. 3.

The fluctuations in the growth of the other experimental cultures are also insignificant when compared with fluctuations in the controls.

These results have possible applications. Since streptomycin seems to have a beneficial effect on growth of the amoeba, the addition of this substance to cultures of *E. nana* might very well prevent a given strain from dying out as a result of an improper balance between the bacterial flora and the amoebae in a culture. Another possibility is that of attempting to get a bacteria-free strain of *E. nana,* since it is able to tolerate such a high concentration of the antibiotic.

SUMMARY

1. *Endolimax nana in vitro* has been subjected to streptomycin sulfate in a range of 20–16360 units per ml for periods ranging from 240–384 hours with no apparent detrimental results upon the amoeba.

2. *E. nana* were subjected to streptomycin residue *in vitro,* in the range of 27–825 units per ml for 288 hours with no inhibitory effects on the amoeba.

3. The possibility of using streptomycin in the cultivation of *Endolimax nana* for the quantitative improvement of the culture is suggested.

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All surviving birds were kept at least four weeks to be sure their recovery was complete. This procedure was then repeated, using a new series (no. 2) of five birds, with the same five strains of *T. gallinae*. Series 3, 4, and 5 were similarly treated.

OBSERVATIONS

Had the results been less clear cut, the determination of the relative virulence of strains of *T. gallinae* by passage through five successive series of pigeons might be open to some question. Under the circumstances, however, certain definite conclusions seem justifiable. A summary of the results is presented in Table 1.

TABLE 1.—*Relative effect of five strains of* Trichomonas gallinae *on five successive series of respectively comparable* Trichomonas-*free homing pigeons*

Series / Strain	1 6 wks. old	2 5½ wks. old	3 5½ mos. old	4 7 mos. old	5 9 mos. old
I Westtown, Penna.	++	++++	++++	++++	++++
II Norristown, Penna.	0	0	0	0	0
III Sumter, S. C.	0	+	0	+	+
IV Chino, Calif.	+++	+++	++++	++	++
V Peregrine falcon	++++	+++	++++	+++	++++

0 = no visible lesions.
+ = very slight caseation, lasting only 2–4 days.
++ = moderate caseation, lasting up to 10 days.
+++ = very severe caseation, remitting just short of death.
++++ = most severe caseation, ending in death.

It is seen that strains II and III produced little or no appreciable effect on their hosts. These were from the commercial pigeon plants where the general practice is to eliminate quickly stock that produces severely cankerous squabs, thus preventing virulent strains from gaining a foothold. In none of the five birds inoculated with strain II was there the slightest gross evidence of pathology. In the birds receiving strain III only three showed canker spots in the mouth, and these areas disappeared in two to four days.

At least one death occurred in the birds inoculated with each of the strains I, IV, and V. Of these, strain IV was least toxic, killing only one pigeon. Two birds with strain IV, however, had severe lesions for over a week, and two were exceedingly cankerous, barely recovering. Strain V nearly killed all five of the pigeons receiving it, succeeding in three, with the other two just managing to come through. Strain I, the worst of all, killed four of the five receiving it. The first bird here was not so badly hit, and recovered. In subsequent tests, not included in these data, strain I proved consistently the most virulent, killing (including the five used here) twelve out of thirteen successive pigeons into which it was put, only the first one escaping. On the average, these twelve birds died at 10.6 days following inoculation. Of the eight birds dying in the present series of twenty-five, the average time of death following inoculation was approximately 9.9 days.

CONCLUSIONS

From the above data it seems obvious that strains (in the present sense) of *Trichomonas gallinae* do vary markedly in their ability to produce visible lesions and/or death in infected pigeons. Strains vary from those which cause little or no visible disturbance to those that constantly produce a high degree of mortality. There also appear to be strains of moderate virulence, in which birds are quite sick, with most of them ultimately recovering.

SUMMARY

1. Twenty-five *Trichomonas*-free homing pigeons (*Columba livia*) were divided into five series of five comparable birds each. Each of five strains of *Trichomonas gallinae* was put into a different bird in the first series of five birds. This was repeated for series 2, 3, 4, and 5.

2. There proved to be a marked variation in strain virulence. Strain II produced no visible pathology. Strain I killed four of the five birds receiving it. Strains III, IV, and V were roughly intermediate between these extremes.

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PROTECTION IN PIGEONS AGAINST VIRULENT *TRICHOMONAS GALLINAE* ACQUIRED BY INFECTION WITH MILDER STRAINS

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INTRODUCTION

It has been established that there are strains of *Trichomonas gallinae* which may differ from one another with respect to their virulence for pigeons (Stabler, 1948). Some strains, though readily becoming established, produce no gross pathology. Others may nearly always be fatal, death being accompanied by extensive caseations of the upper digestive tract, and of the viscera, especially the liver. Some strains appear to be intermediate between these two extremes.

One wonders whether or not infection with one of the generally sub-lethal strains of *T. gallinae* would confer an immunity against one of the highly virulent strains. Such a situation appears to exist in similar flagellate infections. Recovery from an acute *Trichomonas foetus* infection in heifers is generally accompanied by protection against future genital trichomoniasis (see monograph by Morgan, 1944). Likewise, Tyzzer (1932, 1934) showed that infection in young chickens and turkeys with an attenuated strain of *Histomonas meleagridis* resulted in a decided protection against the more virulent strains of the parasite. The present paper presents evidence indicating the existence of a similar situation with respect to *T. gallinae* infection in pigeons.

MATERIAL

The pigeons used in the inoculations were homing pigeons (*Columba livia*) from the writer's *Trichomonas*-free loft. The strains of *T. gallinae* were among those described previously as strains I to V (Stabler, 1948). As potential producers of immunity, strain III (mild) and strain V (fairly severe) were used. Strain I, with a history of great virulence, was the test strain. All infections were established by merely dropping the parasites into the birds' open mouths.

OBSERVATIONS

Experiments were first conducted using the severe strain V for the establishment of protection against the more lethal strain I. Thus, strain V of *T. gallinae* was implanted orally in eight squabs. As seen in Table 1, all birds developed very extensive oral caseation, with two succumbing to the infection. The remaining six recovered completely, and were given oral implantations of the virulent strain I fifty-four days after the initial inoculations with strain V. They had been free of all visible caseation for thirty-one days prior to the second inoculation.

During the next twenty-nine days not one of the six birds showed the slightest sign of caseation, although examined nearly every day. They were then killed and autopsied. Three were completely free of all gross signs of infection, save for the loss of the palatal fringe, which manifestation always accompanies caseation of this area of the palatal region. The other three were likewise free of active trichomoniasis, but showed some liver scarring, indicative of repair following previous caseation in this organ.

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It would appear, then, that recovery from a severe but sub-lethal attack of trichomoniasis due to *T. gallinae* confers an immunity in the pigeon against subsequent attack by a more virulent strain of the same organism.

TABLE 1.—*History of eight 7-week-old* Trichomonas-*free squabs which first received somewhat severe* T. gallinae *strain V, and later highly virulent strain I*

Bird no. / Date	1	2	3	4	5	6	7	8
14.II.47	All birds received orally implanted strain V							
17.II.47	+	+	+	+	+	+	+	++
22.II.47	+++	+++	+++	+++	+++	+++	+++	++++
27.II.47	+	+	+	+	+	+	++++	
9.III.47 to 9.IV.47	0	0	0	0	0	0		
9.IV.47	Six survivors now given strain I							
10.IV.47 to 8.V.47	0	0	0	0	0	0		
8.V.47	All birds lesion free throughout. Old scars as indicated							
killed and posted	liver scars	no scars	no scars	liver scars	liver scars	no scars		

0 = no visible lesions.
+ = very slight caseation.
++ = moderate caseation.
+++ = very severe caseation, remitting just before death.
++++ = most severe caseation, ending in death.

The above experiment was then repeated using the very mild strain III of *T. gallinae* as the protecting strain. Again, eight birds were inoculated orally. Table 2 shows that two birds evidenced very mild, transitory caseation only. The

TABLE 2.—*History of eight 8-week-old (exception, no. 8 = 2 yrs.)* Trichomonas-*free squabs which first received mild* T. gallinae *strain III, and later highly virulent strain I*

Bird no. / Date	1	2	3	4	5	6	7	8
9.V.47	All birds received orally implanted strain III							
12.V.47	0	+	0	0	0	0	0	0
13–21.V.47	0	0	0	0	0	0	0	0
22.V.47	0	0	0	0	0	0	0	+
23.V.47 to 5.VI.47	0	0	0	0	0	0	0	0
5.VI.47	All now given strain I							
6–9.VI.47	0	0	0	0	0	0	0	0
10.VI.47	0	0	0	0	0	0	0	+
13.VI.47	0	0	0	0	0	0	0	++
15.VI.47	0	0	0	0	0	0	0	+
18.VI.47	0	0	0	0	+	0	0	0
19–26.VI.47	0	0	0	0	0	0	0	0
26.VI.47 killed and posted	No evidence of pathology here							much liver scarring and adhesions

0 = no visible lesions.
+ = definite but not severe oral caseation.
++ = severe oral caseation.

other six showed nothing. After all eight birds had been free of caseation for at least thirteen days, all were given oral implantations of the same severe strain I twenty-seven days after receiving strain III.

Only two birds showed any caseation during the next twenty-one days. One had a very fleeting caseous spot on the palate at thirteen days. The other, which was one of the two showing mild caseation with strain III, now developed quite severe oral lesions, from which it eventually recovered. When all birds had been free of caseation for at least seven days they were killed and autopsied (on the twenty-first day after receiving the severe strain). There was not the slightest gross sign of trichomoniasis, past or present, in seven of the birds. The eighth, the same one which developed the extensive caseation after the introduction of the severe strain, showed considerable liver scarring, with some adhesions of the liver to the adjacent peritoneum.

The establishment of mild *T. gallinae* in pigeons, even though there be no gross evidence of pathology, appears likewise sufficient to protect against subsequent damage from infection with a more virulent strain of the same organism.

By way of control, the severe strain I was put into the mouths of thirteen successive *Trichomonas*-free pigeons (Table 3). After the recovery of the first bird,

TABLE 3.—*Control series of* Trichomonas-*free squabs, all of which received oral implantations of the highly virulent* T. gallinae *strain I*

Bird no.	1	2	3	4	5	6	7	8	9	10	11	12	13
Age in weeks at Inoculation	6	5½	22	28	36	25½	7	8	14	7	9	10	14
Result	++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++
Days from inoculation to death	Recovered	10	9	8	9	9	10	7	12	9	7	9	28

++ = severe caseation.
++++ = bird killed by *T. gallinae*.

this strain killed the next twelve in succession. Death occurred on the average 10.6 days after the introduction of the parasite. This strain, then, readily killed birds not protected by recovery from previous infection with milder strains of *T. gallinae*.

SUMMARY

1. A relatively virulent strain of *Trichomonas gallinae* (strain V) was placed orally in eight *Trichomonas*-free squabs. All developed severe oral caseation, from which six recovered. Two died.

2. The six were then given a very virulent strain of *T. gallinae* (strain I) orally. Not the slightest caseation appeared during the next twenty-nine days. On autopsy, three were free of all gross pathology. Three showed old liver scars indicative of former caseation.

3. Eight *Trichomonas*-free birds were now given orally a very mild strain of *T. gallinae* (strain III). Only two showed slight, fleeting caseations.

4. These eight were then given orally the very severe strain (I). One bird developed a small, quickly disappearing oral caseation. Another showed severe oral pathology, from which it recovered. Subsequent autopsy revealed that this

last bird had suffered extensive liver caseation. None of the other seven showed any pathology whatever.

5. By way of control, the very severe strain I was put into the mouths of thirteen successive pigeons. The first one recovered following severe oral caseation; the next twelve died in order, on an average of 10.6 days after inoculation.

6. Recovery in pigeons from a previous infection with a less severe strain of *T. gallinae* appears to be accompanied by some protection against subsequent attack from a very virulent strain of the organism.

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HAEMAPHYSALIS RATTI, A NEW SPECIES OF TICK FROM RATS IN NEW GUINEA AND *HAEMAPHYSALIS KRIJGSMANI,* NEW NAME FOR *HAEMAPHYSALIS NOVAE-GUINEAE* KRIJGSMAN AND PONTO, 1932, PRE-OCCUPIED[1]

GLEN M. KOHLS
United States Public Health Service

In the course of examining New Guinea rats for trombiculid mites and other ectoparasites during wartime scrub typhus studies conducted in 1943 and 1944 by the United States of America Typhus Commission, the writer found several specimens of the new species of *Haemaphysalis* here described. The assistance of Mr. Ellis Troughton, Mammalogist, The Australian Museum, Sydney, in identifying the rats is gratefully acknowledged.

Haemaphysalis ratti n. sp.

Male (Fig. 1, A–H)

Body: Length from tips of palpi to posterior margin (2 specimens), 1.98[2] and 2.01. Width, 0.93 and 0.96. Long oval, widest at about the fourth coxae.

Capitulum: Length from tips of palpi to tips of cornua, 0.31; length of basis, 0.144; width, 0.245. Basis broad, lateral margins convergent posteriorly, posterior margin straight. Cornua short and pointed. Palpi with article 2 very salient laterally and much longer than article 3. In ventral view basis flattened, broadly rounded posteriorly. Palpal article 2 with 3 or 4 broad hairs on the internal margin; article 3 with a short retrograde spur.

Hypostome: Clavate, with a large corona. Dentition 4/4. Denticles about equal in size, with about 8 in each of the outermost files and 6 or 7 in the inner files. Length about 0.134.

Scutum: Length from tips of scapulae to posterior margin (2 specimens), 1.74 and 1.80. Lateral grooves long, deep, and continuing across 3 festoons. Cervical grooves short, deep, and convergent anteriorly, then shallow and divergent posteriorly. Punctations distinct, moderate in size, and evenly distributed, except in posterior pseudoscutal area, where they are fewer in number.

Legs: Coxa I with a short, rounded, internal spur; II and III each with a very short, broad spur; IV with one bluntly pointed spur. Trochantal spurs and ventral spurs on tarsi absent. Length of tarsus I, 0.297; metatarsus, 0.182. Length of tarsus IV, 0.278; metatarsus, 0.182.

Spiracular Plate: Suboval, greatest dimension about 0.270.

Genital Aperture: Between coxae II.

Female (Fig. 2, A–H)

Body: Length, unfed specimen, from tips of palpi to posterior margin, 2.40; width, 1.08. Long oval, widest at about the fourth coxae. Fully fed specimens are long oval, and about 7.0 long by 4.5 wide. Marginal grooves distinct, beginning at about coxae II and continuing across 2 festoons. Festoons about as wide as long.

Capitulum: Length from tips of palpi to tips of cornua, 0.43; length of basis, 0.192; width, 0.374. Basis nearly twice as broad as long, lateral margins convergent posteriorly, posterior margin straight, cornua short and pointed. Porose areas shallow, oval, well separated. Palps as in the male, dorsally and ventrally.

Hypostome: Clavate, with a small corona. Dentition 4/4. Principal denticles all about equal in size and with 8 to 10 in each file. Length, about 0.224.

Scutum: Measurments of 11 specimens range from 0.84 to 0.96 in length and from 0.63 to 0.72 in width. Broadly rounded posteriorly. Cervical grooves long, distinct. Punctations moderate in number; less numerous and smaller in the area between and posterior to the cervical grooves.

Legs: Essentially as in the male. Length of tarsus I, 0.374; metatarsus, 0.201. Length of tarsus IV, 0.336; metatarsus, 0.211.

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[1] From the Rocky Mountain Laboratory (Hamilton, Montana), National Institute of Health.

[2] All measurements in millimeters.

Spiracular Plate: Suboval, greatest dimension about 0.288.
Genital Aperture: Opposite the interval between coxae II and III.
Holotype: Male, 21171, Dobodura area of British New Guinea, from *Rattus* sp. near *mordax,* May 4, 1944.

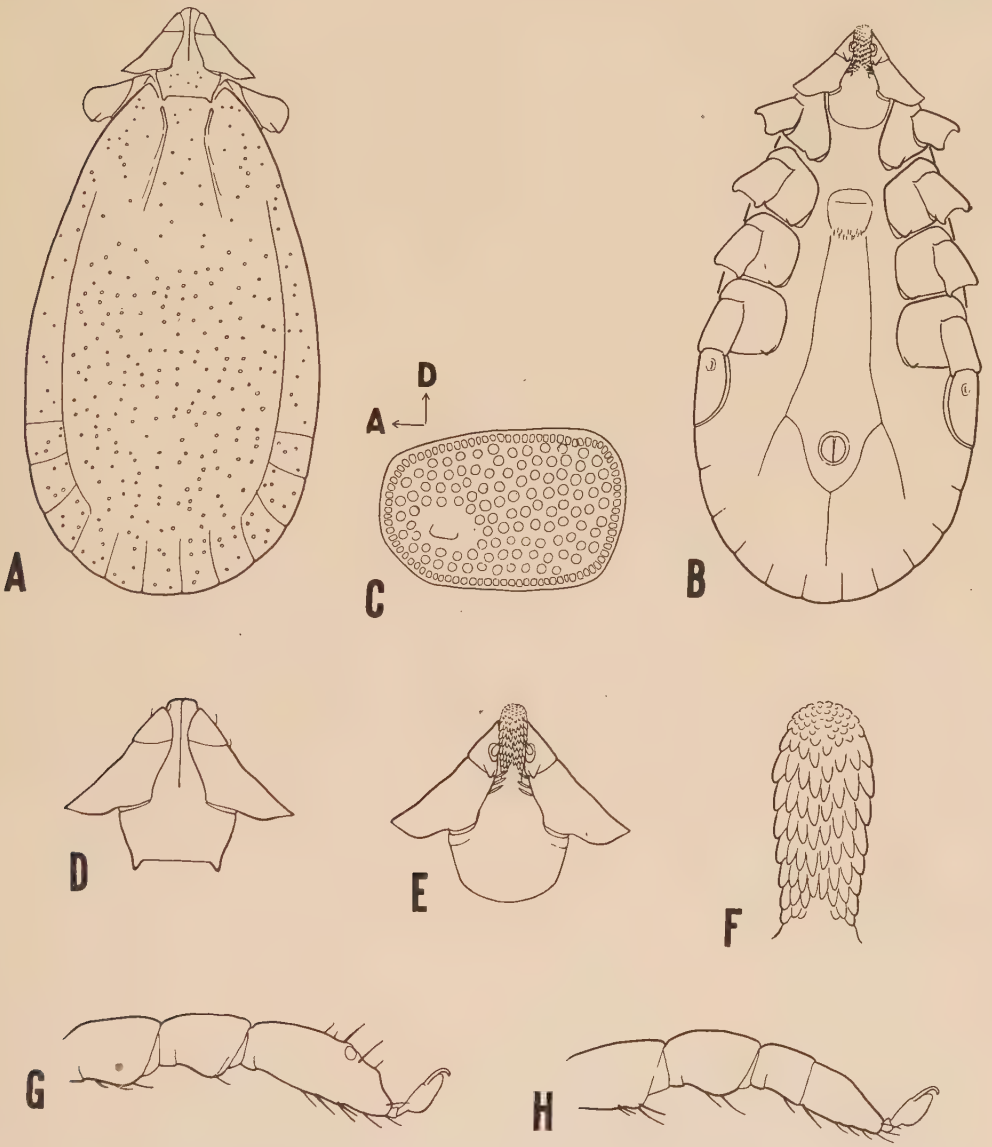

FIG. 1. *Haemaphysalis ratti* n. sp.—Male. A. Capitulum and scutum, dorsum. B. Capitulum and body, venter. C. Spiracular plate. D. Capitulum, dorsum. E. Capitulum, venter. F. Hypostome. G. Tarsus and metatarsus, leg I. H. Tarsus and metatarsus, leg IV.

Allotype: Female, same data as holotype.
Paratypes: All from the Dobodura area of British New Guinea; 1 female, 20947, from *Rattus gestri,* December 4, 1943; 1 male, 1 female, 21174, from *Rattus* sp. near *mordax,* April 12, 1944; 1 female, 21175, from *Rattus mordax,* May 12, 1944; 4 females, 21180, from *Rattus mordax,* May 19, 1944; 1 female, 21183, from *Rattus mordax,* July 11, 1944; 1 female, 21184, from *Rattus mordax,* July 20, 1944, and 1 female, 21189, from *Rattus mordax,* May 10, 1944.

Types are deposited as follows: Holotype, allotype, and paratypes in the Rocky Mountain Laboratory, Hamilton, Montana; 1 paratype female (21175), United States National Museum, Washington, D. C.; 1 paratype female (21183), British Museum (Natural History), London, England; 1 paratype female (21184), The Australian Museum, Sydney, Australia.

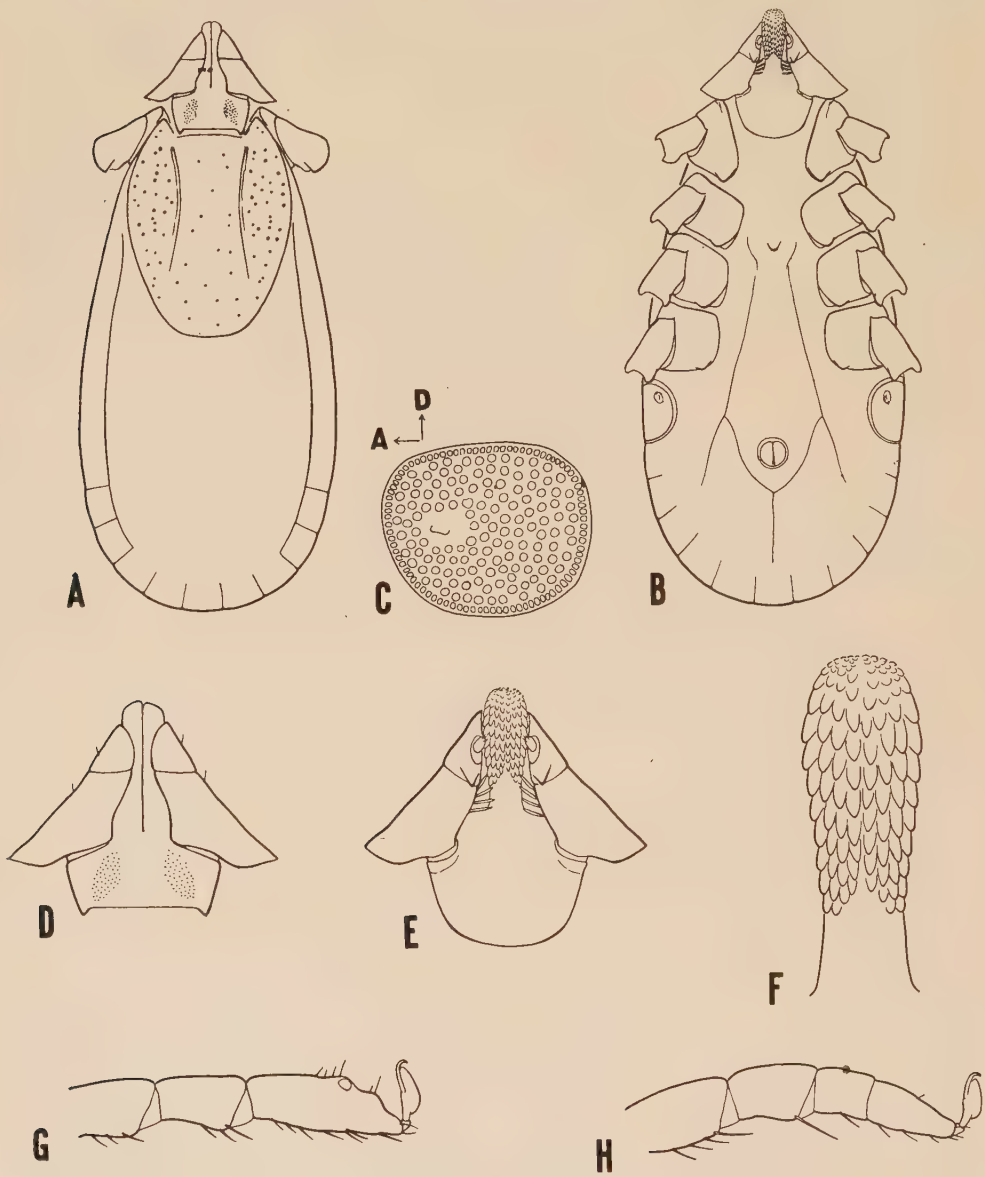

FIG. 2. *Haemaphysalis ratti* n. sp. Female. A. Capitulum, scutum and body, dorsum. B. Capitulum and body, venter. C. Spiracular plate. D. Capitulum, dorsum. E. Capitulum, venter. F. Hypostome. G. Tarsus and metatarsus, leg I. H. Tarsus and metatarsus, leg IV.

In some respects *H. ratti* resembles *H. humerosa* Warburton and Nuttall, 1909, a species previously known from Australia, but which the writer found to be common on bandicoots, *Echymipera cockerelli,* in the Dobodura area. The new species is readily distinguished from *humerosa* in that it is not so markedly elongate, the denti-

tion of the hypostome of both sexes is 4/4 instead of 5/5, the coxae are not as broad anteroposteriorly as in *humerosa,* and by other characters including those of the scutum, basis capituli, and palps.

Haemaphysalis krijgsmani, new name.

Hirst (1914) described *Haemaphysalis novae-guineae* from specimens taken from kangaroos, and from *Peramales* sp. and *Hydromys* sp. at Sattelberg, Huon Gulf, German New Guinea, 1911, by Prof. F. Forster. In their monograph on the genus *Haemaphysalis,* Nuttall and Warburton (1915) reduced *novae-guineae* to a variety of *spinigera* Neumann, 1897. Krijgsman and Ponto (1932) described a new species which they named *Haemaphysalis novae-guineae* from specimens taken from wild pigs at Merauke in Dutch New Guinea, apparently overlooking the earlier use of the name by Hirst. Since *novae-guineae* Krijgsman and Ponto, 1932, is pre-occupied by *novae-guineae* Hirst, 1914, in the genus *Haemaphysalis,* the new name, *H. krijgsmani,* is here proposed for the former.

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ON THE DEMONSTRATION OF HYALURONIDASE IN CERCARIAE OF *SCHISTOSOMA MANSONI**

MILTON D. LEVINE, RAY F. GARZOLI, ROBERT E. KUNTZ, AND JOHN H. KILLOUGH
Naval Medical Research Institute, National Naval Medical Center, Bethesda 14, Maryland

Parasitologists have for years spoken and written of "histolytic" and proteolytic enzymes or ferments as a mechanism whereby cercariae penetrate through the ground substance of skin. To date this mechanism has remained in the realm of speculation with the exception of the work by Davis (1936) who demonstrated the presence of a "lytic" material derived from macerated cercariae of *Diplostomum flexicaudum*. This material was shown to produce swelling and distintegration of tadpole skin and muscle.

Duran-Reynals (1942) has recently reviewed the properties of the so-called ground substance and Meyer (1941) has demonstrated the presence of hyaluronic acid, a mucopolysaccharide, in pig skin. Hyaluronidase, an enzyme capable of disaggregating and depolymerizing hyaluronic acid, has been demonstrated in numerous bacterial organisms, and has been shown to be a mechanism whereby these organisms spread in skin (Duran-Reynals, 1942; Meyer et al, 1941). This fact suggested the possibility that this enzyme might well constitute a mechanism of cercarial penetration into the host.[1]

METHODS

Snails, *Australorbis glabratus* (Say) infected with *Schistosoma mansoni* were placed in small glass bowls containing "conditioned tap water,"[2] and subjected to the heat of an 100-watt incandescent lamp placed at a distance of 1 foot from the bowls for a 3-hour period. By this means the temperature of the water was raised to about 35° C. This elevation above the usual aquarial temperature of 23–25° C resulted in emergence of large numbers of cercariae, varying between 20,000 to 60,000 organisms (Kuntz, 1947). Cercariae used in this study had been emerging for from 10 days in some snails to 4 months in others.

Substrate (3 mg of sodium hyaluronate) was added to the suspension of cercariae in 60 ml. of "condition tap water," and the preparation was incubated at room temperature (25° C) for 18 hours. Water was then evaporated off under high vacuum. This required approximately 36 hours during which time the temperature was stabilized at just above freezing. The dry material was taken up in 1 ml of 0.05 M phosphate buffer having a pH of 6.18. Insoluble particles were removed by gentle centrifugation, and the supernatant solution assayed for hyaluronidase.

Control solutions consisted of "conditioned tap water" containing large amounts of snail feces but very few cercariae. Immediately after cercarial emergence the

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* The opinions or assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

[1] Macerated cercariae of *S. mansoni* were shown to produce marked spreading of Evans blue in rabbit skin in previous work done in this laboratory (R.E.K.–J.H.K.)

[2] "Conditioned tap water" refers to ordinary tap water which has stood in the laboratory for at least 1 week.

supernatant suspension was decanted from the snail feces and centrifuged to remove cercariae. The water was returned to the dish with snail feces, and then treated exactly the same as the cercarial suspension. A control was used for each sample of cercariae assayed.

The sodium salt of hyaluronic acid was prepared by the method of Meyer (1947) from freshly collected human umbilical cords stored in acetone. The preparation was completely water-soluble and gave a clear viscous solution. A 0.25 per cent solution of sodium hyaluronate was prepared in 0.05 M phosphate buffer at a pH of 6.18 for use as substrate.

FIG. 1. Semi-micro viscometer used for hyaluronidase assay.

Enzyme activity was determined by the viscometric method for the assay of hyaluronidase (Madinaveitia and Quibell, 1940). A special viscometer was designed to use small amounts of material and yet give reproducible results over a wide range of viscosity values. Fig. 1[3] shows this instrument.

1.0 ml. of substrate and 0.3 ml. of enzyme solution or control solution were mixed in the viscometer and the initial flow time determined immediately. The change in

[3] The 12-mm glass tube is 25 cm long. The capillary tubing has a bore of 0.75 mm and is 70 cm long. The diameter of the spiral is 35 mm. The capacity of the bulb in the capillary is approximately 0.3 ml. Viscosity of as little as 0.9 ml of solution can be determined. The advantage of this viscometer design is that long flow times can be obtained with relatively large capillary bore. Small particles that may be present in the solutions, therefore, introduce very small errors.

relative viscosity of the enzyme-substrate mixture was followed for a period of 100 minutes in each case.

The hyaluronidase activity of each cercarial suspension is plotted in Fig. 2. Enzyme activity is a function of the reduction of the relative viscosity of the enzyme-substrate mixture with time (Madinaveitia and Quibell, 1940). It is evident that each cercarial suspension prepared with sodium hyaluronate shows considerable activity although we found no relationship between activity and number of cercariae. If sodium hyaluronate is not included in the suspension during the incubation period the hyaluronidase activity is markedly diminished or absent. By dividing equally sev-

RESULTS

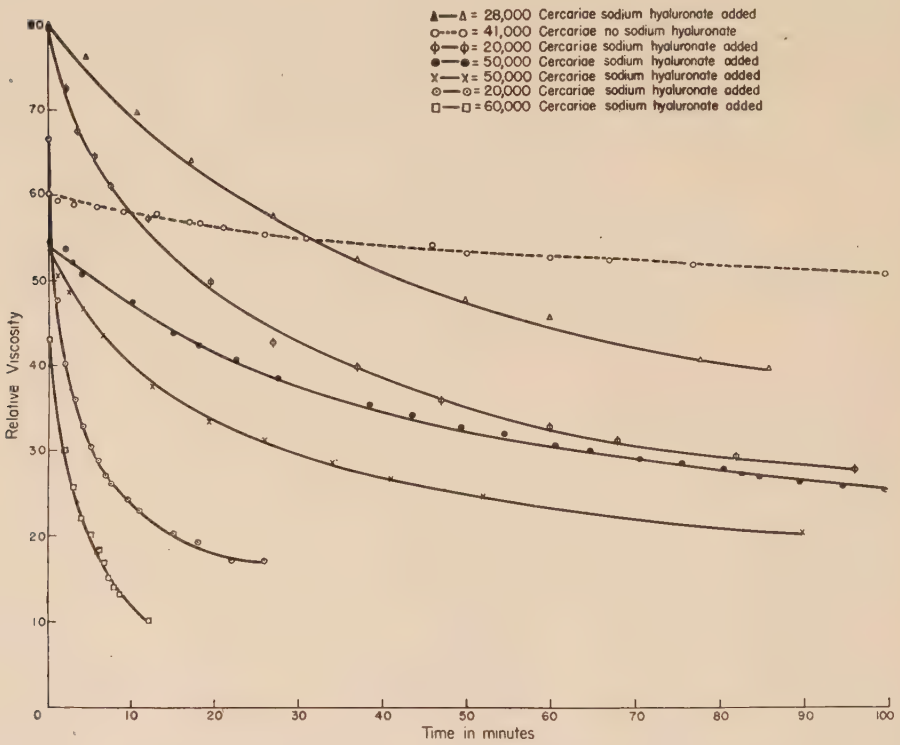

FIG. 2. Hyaluronidase activity curves of cercariae of *S. mansoni* incubated at 25° centigrade for a period of 18 hours. The change in relative viscosity of the sodium hyaluronate substrate-cercarial mixture with time is a function of enzyme activity.

eral cercarial suspensions and incubating one portion with a small quantity of the substrate, and the other portion without substrate we were able to demonstrate a marked difference in activity. This phenomenon has been shown to hold true for some bacterial cultures (McClean and Hale, 1941).

Our control solutions were consistently negative which eliminates the possibility that bacteria were responsible for the hyaluronidase activity.

DISCUSSION

Duran-Reynals (1942) has discussed at length the invasive properties of bacterial organisms in terms of depolymerization of mucoid ground substance of connective

tissues by hyaluronidase, the bacteria then "spreading" through a relatively fluid medium. The demonstration of the ability of *S. mansoni* cercariae to produce this enzyme suggests the occurrence of this mechanism in cercarial penetration although we do not think this explains the entire phenomenon. We have made no attempt to identify collaginase or proteolytic enzymes in our suspensions but they may well be present.

The lack of correlation between enzyme activity and cercarial concentration requires explanation. Over the course of the experiment as snails died new snails were added. Thus the cercarial yield varied not only as to the number of organisms but as to the duration of the infection in the snails, and by virtue of the forced emergence of cercariae by heat, the age of the organisms at the time of emergence. These factors are beyond laboratory control where large numbers of cercariae are required. One would expect the yield showing the least enzyme activity to be the least infective. We have not as yet tested this postulate, but preliminary observations indicate that the number of adult worms recoverable from mice exposed to identical numbers of cercariae derived from our snail colony varies considerably. Whether this variation be a function of hyaluronidase activity of cercariae remains to be demonstrated.

In vivo inhibitors of this enzyme system may well be studied as a means of prophylaxis against schistosome infections. Thus far we have tried sodium salicylate with negative results.

SUMMARY AND CONCLUSIONS

1. Cercariae of *S. mansoni* produce hyaluronidase when incubated for 18 hours at 25° C if sodium hyaluronate is included in the cercarial suspensions.

2. We were unable to demonstrate a relationship between the number of cercariae and hyaluronidase activity.

3. This may be a mechanism whereby cercariae penetrate the skin of the host.

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RESEARCH NOTES

THE CULTIVATION OF *TRYPANOSOMA CRUZI* IN DIALYSATE MEDIUM

M. Lwoff (1940, Monographies de L'Institut Pasteur) has summarized the work on growth requirements of TRYPANOSOMIDAE *in vitro*. As a result of these investigations *Trypanosoma cruzi* and several other species which are transmitted to mammals by an invertebrate host have been found to require vitamins B_1 and C, dextrose, iron-containing constituents of hemoglobin and certain unidentified ingredients of serum. It appears that progress in identification of the growth requirements might be facilitated by the use of simpler media than those thus far employed. Accordingly we have investigated growth of *T. cruzi* in a dialysate medium.

This medium is prepared in loops of cellophane tubing by suspending the loops in the overlay of a diphasic blood agar medium which is used for the routine cultivation of the organism. The stock medium has supported excellent growth of *T. cruzi* over a period of several years and is prepared in the following manner: The blood agar is made essentially as recommended by Senekjie (1943, Am. J. Trop. Med. **23**: 523–531), and consists of bacto-beef (Difco), 50 gm; neopeptone (Difco), 20 gm; bacto-agar (Difco), 20 gm; NaCl, 5 gm; distilled water, 1000 ml; and defibrinated rabbit's blood, 100 ml. The Locke's overlay contains NaCl, 8 gm; KCl, 0.2 gm; $CaCl_2$, 0.2 gm; KH_2PO_4, 0.3 gm; dextrose, 2.5 gm; and distilled water, 1000 ml. In the preparation of the dialysate medium 10 ml of the stock base is slanted in 25 × 200-mm test tubes and overlaid with 10 ml Locke's solution per tube. The loops are made from 8-inch lengths of ¾-inch (flat width) cellophane tubing. Each loop is tied to a short glass tube by string and the loop suspended in the overlay of the diphasic medium by means of rust-resistant wire. This arrangement is patterned after that used by Rees, Reardon, Johnson, and Mayfield (1945, J. Parasitol. **31** (Suppl.) : 7) in the cultivation of *Endamoeba histolytica*. The loop assembly is illustrated in Fig. 1.

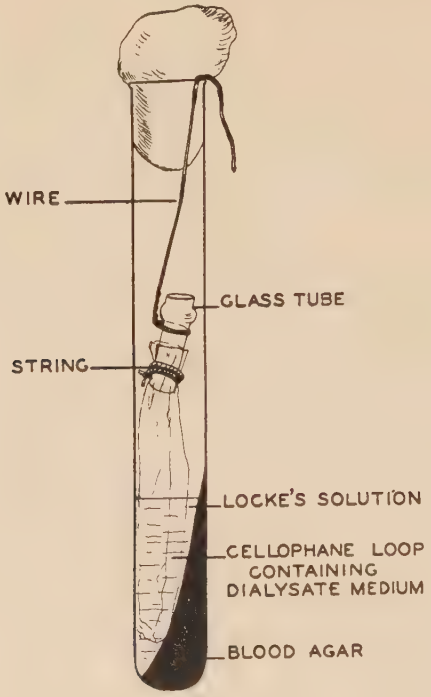


FIG. 1.

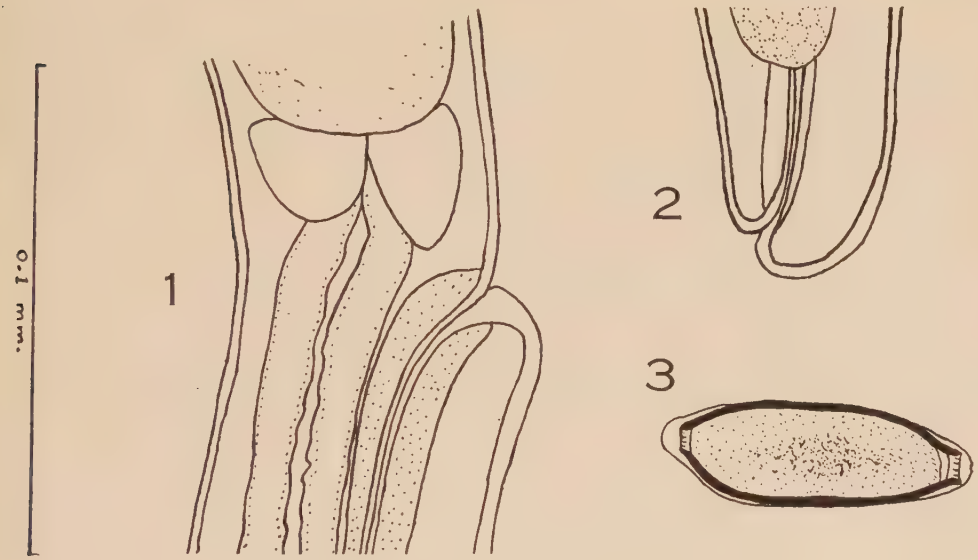
To guard against leaks in the loops and bacterial contamination of the medium, loops are soaked overnight and tested under water with air pressure equivalent to 10 cm Hg. They are then supported within test tubes and sterilized by autoclaving. Sterile Locke's solution is added in 7-ml amounts per loop and the loops transferred to the sterile diphasic medium.

T. cruzi has been maintained in this dialysate medium at a temperature of 24–25° C for more than a year. Serial transfers have been made at biweekly intervals from an inoculum of approxi-

mately one ml of loop fluid containing active organisms. This demonstrates that *T. cruzi* may be grown without proteins or other non-dialyzable substances. Growth as determined microscopically has been good and the viability of the organisms has not decreased.—ELEANOR JOHNSON TOBIE AND CHARLES W. REES, *Division of Tropical Diseases, National Institute of Health, United States Public Health Service, Bethesda, Maryland.*

A NEW CAPILLARID NEMATODE FROM THE SPINY DOGFISH, *SQUALUS ACANTHIUS*

During the examination of a single dogfish, *Squalus acanthius,* from Galveston Bay three female worms of the genus *Capillaria* were found in the small intestine. A search of the literature reveals that two species of the genus have been described from Selachians. These two species, *C. carcharini* MacCallum, 1925, and *C. spinosa* MacCallum, 1926, are known only from the eggs. *C. carcharini* was described from eggs found in the tissues of the mouth and nasal region of *Carcharinus lamia* (Raf.) and *C. spinosa* from eggs found in the tissues of the branchial region of *Carcharinus milberti* (Mueller and Henle). MacCallum (1925, Proc. U.S.N.M., **67**, Art. 16, pp. 1–2) described the eggs of *C. carcharini* as 0.100 mm long by 0.005 mm wide. This is



FIGS. 1–3. *Capillaria hathawayi.* FIG. 1. Vulva. FIG. 2. Cauda of female. FIG. 3. Egg.

obviously a typographical error since his figure with its accompanying scale shows that the egg is about 0.05 mm wide. Baylis and Jones (1933, J. Marine Biol. Assn. United Kingdom, n.s., **18**: 627–634) recorded a *Capillaria* sp. from the spiral valve of *Scylliorhynchus catalus,* but did not describe the single female worm encountered by them. The eggs of the worms from the dogfish from Galveston Bay are much smaller than those of *C. carcharini* or *C. spinosa* and differ from these species in having a smooth outer shell. It has not been possible to refer this material to any of the other species described from fishes, and it is thought, therefore, that these worms represent a new species which is named in honor of Dr. E. S. Hathaway of Tulane University.

Capillaria hathawayi n. sp.

Specific diagnosis: Body smooth. Bacillary lines absent.

Female: 25.0 to 31.9 mm long; 60 to 65 μ wide just posterior to vulva; maximum width 78 to 82 μ. Vulva without appendage and located just posterior to termination of esophagus, 6.6 to 7.7 mm from anterior end. Posterior labium slightly salient. Vulva divides body 1 : 2.8 to 1 : 4.2. Eggs 56 to 65 μ long by 26 to 28 μ wide; inner shell forms slight collar for plug at each end; outer shell smooth. Anus subterminal.

Male: Unknown.

Host: Squalus acanthius L.

Habitat: Small intestine.

Locality: Galveston Bay, Gulf of Mexico.

Type: U. S. Natl. Mus. Helminth. Coll. No. 46333.

Thanks are due to Dr. Asa C. Chandler under whose direction this work was done.—CLARK P. READ, *Dept. of Biology, Rice Institute, Houston, Texas.*

ON THE LIFE CYCLE AND SYSTEMATIC POSITION OF THE ASPIDOGASTRID TREMATODE, *COTYLOGASTER OCCIDENTALIS* NICKERSON, 1902

During the summers of 1935 and 1936, 144 *Cotylogaster occidentalis* parasites of various sizes, but mostly large, were recovered from 7697 *Goniobasis* snails from the Bass Island Region of Lake Erie. This represents approximately a 2 per cent infestation. The larger parasites were mature in that they contained numerous eggs in which there were viable embryos. All observations made upon the immature and mature parasites indicate that this species undergoes direct development within the snail host. Nickerson (1902, Zool. Jahrb., Syst. **15**: 597–624) first reported this parasite as infesting the Sheephead, *Aplodinotus grunniens.* Kelly (1927, Proc. Iowa Acad. Sci. **33**: 339) reported them as infesting the clam, *Lampsilis luteola.* The author examined 22 Sheepheads from Lake Erie and one large fish was found to contain 3 specimens of *C. occidentalis* in the caudal end of its intestine. There was also evidence in its stomach that it had eaten *Goniobasis* snails.

Feeding experiments were started in which the larger parasites recovered from crushed snails were forcibly fed to Rock Bass, *Ambloplites rupestris.* The parasites passed through the Rock Bass without becoming attached and they seemed hopelessly entrapped in mucous at the time they were passed. Later, experimental feeding was done with small Sheepheads since none of the small ones had been found to harbor the parasite and none of them showed any evidence of having eaten *Goniobasis* snails. During a five day period, July 25 to 29, inclusive, 1936, each of five small Sheepheads taken from Lake Erie were forcibly fed, one each day, five large specimens of *C. occidentalis* which had been recovered from crushed *Goniobasis* snails. Following the forced feeding of the five parasites the fish were placed in a floating and anchored live box and left there, without food, until August 3. At this time the fish were opened and each was found to contain five specimens of *C. occidentalis* in the caudal end of its intestine. It is assumed that these five parasites were present in each fish as a result of their having been forcibly fed to the fish.

While it was thus possible to infest the Sheephead experimentally with *C. occidentalis* it must be remembered that most of the specimens of this parasite, which were recovered from crushed snails, contained eggs with viable embryos and they, therefore, are not dependent upon a vertebrate host for continued existence.

This added knowledge of the life cycle of an aspidogastrid trematode provides further evidence that the Subclass ASPIDOGASTREA, as proposed by Faust and Tang (1936, Parasitology **28**: 487–501) and supported by Williams (1942, J. Parasitol. **28**: 467–474) and by Van Cleave and Williams (1943, J. Parasitol. **29**: 127–130), should become recognized as a valid Subclass of the Class TREMATODA. The above statement is pertinent since the 1947 revision of "Zoological Names, A List of Phyla, Classes, and Orders" prepared for Section F of the American Association for the Advancement of Science lists the Suborder ASPIDOCOTYLEA Burmeister 1856 (ASPIDOCOTYLINA) in the Subclass DIGENEA Carus 1863 (DIGENIA), and some investigators have followed this scheme in recent publications.—E. EUGENE DICKERMAN, *Department of Biology, Bowling Green State University, Bowling Green, Ohio.*

USE OF VERNACULAR NAMES, SEA-LION AND FUR SEAL

In a recent paper by Wardle, McLeod, and Stewart (1947, J. Parasitol. **33**(4): 319–330) the authors refer three times (pages 320, 321, and 324) to a host of their new cestode genus *Cordicephalus* as "the sea-lion (*Callorhinus ursinus*)." However, the vernacular name of *Callorhinus ursinus* is fur seal and the vernacular name sea-lion is used in Alaska exclusively for *Eumetopias jubata.* These usages are accepted by professional mammalogists. It seems desirable that the confusion produced by the accidental misapplication of an established vernacular name be corrected.—VICTOR B. SCHEFFER, *U. S. Fish and Wildlife Service, Seattle, Washington.*

IN MEMORIAM

RUDOLF W. GLASER (1888–1947)

The field of parasitology and insect pathology lost an investigator of distinction in the death on September 4, 1947 of Rudolf W. Glaser. His studies on insects dealt with the causes of their parasitisms, and their reactions as hosts. All their "parasites" interested him,—viruses, fungi, bacteria, "symbionts" or "Rickettsiae," protozoa, and nematodes, in relationships that ranged from symbiosis to fatal parasitosis. Desiring uncontaminated disease agents for his investigations, he devised methods of purifying and culturing a variety of species, not infrequently finding with skill the subtler nutritional requirements required by fastidious, hard-to-grow microorganisms. Such studies were extended to include the bacteria-free rearing of free-living protozoa, and host insects, as well as parasites of mammals. As a result of his work on a nematode inhabiting the grub of the Japanese beetle, he was able, in course, to render assistance in the control of the host pest, and later to bring the nematode into successful sterile culture.

An early concern was with wilt disease of the gypsy moth, *Porthetria dispar,* the cause of which he and J. W. Chapman in 1913[1] correctly ascribed to a filterable virus. This was following a 1912[2] report by the same authors that a bacterial species was the etiological agent. The correction of this error, with G. F. White's 1913 demonstration of sacbrood in bees being due to a filterable virus, "clarified the confusion which had enveloped the cause of this group of diseases in insects, permitting the virologists to include one more form of life in the list of those experiencing virus diseases" (Steinhaus[3]). The effect on the young investigator was two-fold. He promptly established a rigidity of standards as to what constituted reasonable proof of etiologic and biologic relationship (one recalls a somewhat similar episode in the hog cholera bacillus of Theobald Smith), a characteristic that is reflected in the durability of all of his later work; and he became an early and persistent investigator of the filterable viruses, with special reference to insects. The best review of the status of this field up to 1928 is the one he wrote,[4] but this did not end his contributions to it.[5]

During his studies on immunity in insects, Glaser's interest was attracted to their intracellular inhabitants. He comprehensively reviewed the problem of these "symbionts" and "Rickettsiae," and showed that in two roach species, *Periplaneta americana* and *Blattella germanica,* "the micro-organisms discussed were indeed bacteria."

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[3] Insect Microbiology, Comstock Publishing Co., Ithaca, N. Y., 1946, p. 413. In listing 30 of Glaser's articles (including those of joint authorship) in his comprehensive 89 pages of "References," Steinhaus makes him the most cited author in the field of insect microbiology; next come S. Metalnikov, and A. Paillot.

[4] Virus diseases of insects, Chap. VIII (pp. 279–332) in Filterable Viruses, T. M. Rivers, Editor, Williams and Wilkins Co., Baltimore.

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Locality: Galveston Bay, Gulf of Mexico.

Type: U. S. Natl. Mus. Helminth. Coll. No. 46333.

Thanks are due to Dr. Asa C. Chandler under whose direction this work was done.—
CLARK P. READ, *Dept. of Biology, Rice Institute, Houston, Texas.*

ON THE LIFE CYCLE AND SYSTEMATIC POSITION OF THE ASPIDOGASTRID TREMATODE, *COTYLOGASTER OCCIDENTALIS* NICKERSON, 1902

During the summers of 1935 and 1936, 144 *Cotylogaster occidentalis* parasites of various sizes, but mostly large, were recovered from 7697 *Goniobasis* snails from the Bass Island Region of Lake Erie. This represents approximately a 2 per cent infestation. The larger parasites were mature in that they contained numerous eggs in which there were viable embryos. All observations made upon the immature and mature parasites indicate that this species undergoes direct development within the snail host. Nickerson (1902, Zool. Jahrb., Syst. **15**: 597–624) first reported this parasite as infesting the Sheephead, *Aplodinotus grunniens.* Kelly (1927, Proc. Iowa Acad. Sci. **33**: 339) reported them as infesting the clam, *Lampsilis luteola.* The author examined 22 Sheepheads from Lake Erie and one large fish was found to contain 3 specimens of *C. occidentalis* in the caudal end of its intestine. There was also evidence in its stomach that it had eaten *Goniobasis* snails.

Feeding experiments were started in which the larger parasites recovered from crushed snails were forcibly fed to Rock Bass, *Ambloplites rupestris.* The parasites passed through the Rock Bass without becoming attached and they seemed hopelessly entrapped in mucous at the time they were passed. Later, experimental feeding was done with small Sheepheads since none of the small ones had been found to harbor the parasite and none of them showed any evidence of having eaten *Goniobasis* snails. During a five day period, July 25 to 29, inclusive, 1936, each of five small Sheepheads taken from Lake Erie were forcibly fed, one each day, five large specimens of *C. occidentalis* which had been recovered from crushed *Goniobasis* snails. Following the forced feeding of the five parasites the fish were placed in a floating and anchored live box and left there, without food, until August 3. At this time the fish were opened and each was found to contain five specimens of *C. occidentalis* in the caudal end of its intestine. It is assumed that these five parasites were present in each fish as a result of their having been forcibly fed to the fish.

While it was thus possible to infest the Sheephead experimentally with *C. occidentalis* it must be remembered that most of the specimens of this parasite, which were recovered from crushed snails, contained eggs with viable embryos and they, therefore, are not dependent upon a vertebrate host for continued existence.

This added knowledge of the life cycle of an aspidogastrid trematode provides further evidence that the Subclass ASPIDOGASTREA, as proposed by Faust and Tang (1936, Parasitology **28**: 487–501) and supported by Williams (1942, J. Parasitol. **28**: 467–474) and by Van Cleave and Williams (1943, J. Parasitol. **29**: 127–130), should become recognized as a valid Subclass of the Class TREMATODA. The above statement is pertinent since the 1947 revision of "Zoological Names, A List of Phyla, Classes, and Orders" prepared for Section F of the American Association for the Advancement of Science lists the Suborder ASPIDOCOTYLEA Burmeister 1856 (ASPIDOCOTYLINA) in the Subclass DIGENEA Carus 1863 (DIGENIA), and some investigators have followed this scheme in recent publications.—E. EUGENE DICKERMAN, *Department of Biology, Bowling Green State University, Bowling Green, Ohio.*

USE OF VERNACULAR NAMES, SEA-LION AND FUR SEAL

In a recent paper by Wardle, McLeod, and Stewart (1947, J. Parasitol. **33**(4): 319–330) the authors refer three times (pages 320, 321, and 324) to a host of their new cestode genus *Cordicephalus* as "the sea-lion (*Callorhinus ursinus*)." However, the vernacular name of *Callorhinus ursinus* is fur seal and the vernacular name sea-lion is used in Alaska exclusively for *Eumetopias jubata.* These usages are accepted by professional mammalogists. It seems desirable that the confusion produced by the accidental misapplication of an established vernacular name be corrected.—VICTOR B. SCHEFFER, *U. S. Fish and Wildlife Service, Seattle, Washington.*

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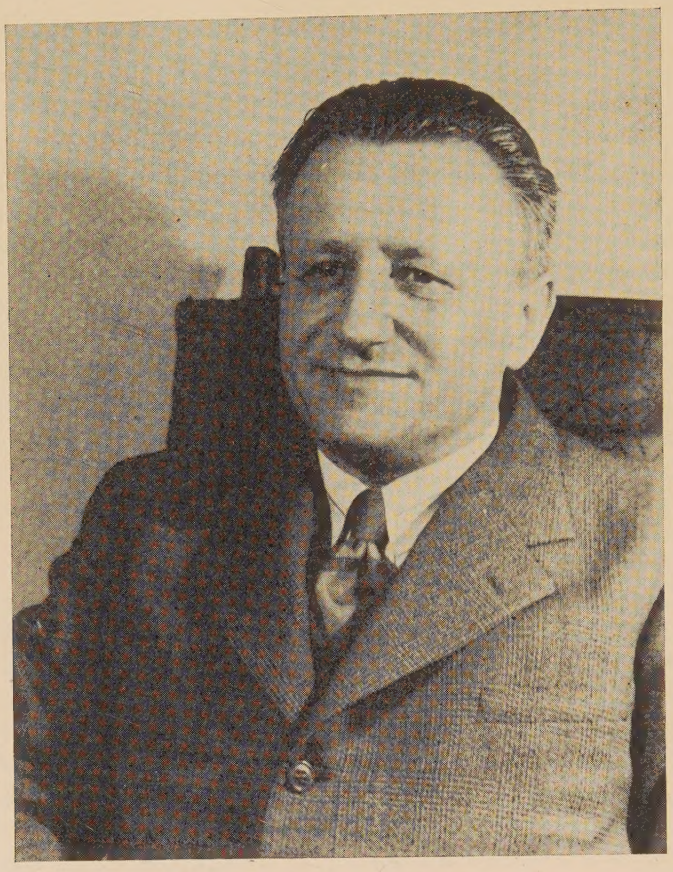

RUDOLF W. GLASER (1888–1947)

Also that they were transmitted from one generation to another through the ova, and that treatment eliminating such intracellular organisms from the host prevented the development or caused the regression of the female sex glands.[6]

In 1918[7] while experimenting with grasshopper diseases and strains of d'Herelle's *Coccobacillus acridiorum,* Glaser noted that many species of Acridians developed an immunity toward bacterial infections. He showed that this immunity could be experimentally produced in healthy insects. This suggested the possibility of inoculating grasshoppers with the intestinal contents of flies containing herpetomonads and bacteria in order to obtain a pure culture of the protozoa. *Herpetomonas muscae-domesticae* was sterilized in this way, and grown, although not at the time very successfully. Later he attacked the problem again using 4th or 5th stage larvae of the silkworm, *Bombyx mori,* for the "screening" host, and by this means freed *H. muscae-domesticae* from accompanying intestinal flora and grew it serially in culture at will.[8]

These attempts led to a study of conditions permitting the bacteria-free growth

[6] Arch. Path. **9**: 71–96, 557–576, 1930; J. Exper. Med. **51**: 59–82, 903–907, 1930; Science **74**: 243, 1931; J. Parasitol. **32**: 483–489, 1946.

[7] Ann. Entom. Soc. America **11**: 19–42; Psyche **25**: 39–46.

[8] J. Parasitol. **8**: 99–108, 1922; Am. J. Trop. Med. **6**: 205–219, 1926.

of free-living protozoa. (The project had another objective, namely that such sterilely grown protozoa might permit the growth in vitro of certain viruses, but this objective was not attained.) Successful cultures eventually included several species, among them *Paramecium caudatum.* The latter proved surprisingly refractory to being freed from its contaminants or growing without live organisms for food; the methods by which he succeeded with it furnished good illustration of Glaser's resourcefulness as an investigator. Following these came simplified methods[9] of purification and culture without contaminants of *Tritrichomonas foetus,* a bovine pathogen, and partial success with *Balantidium coli* from swine.

It was in 1930[10] that Glaser reported, with H. Fox, a nematode (named *Neoaplectana glaseri* by Steiner[11]) parasitizing the grub of the Japanese beetle, *Popillia japonica.* This helminth engaged a good deal of Glaser's attention for several years. It is an especially interesting form in that while evidencing readiness to parasitize and kill beetle grubs, it also showed the ability to undergo multiplication within beetle grub cadavers.[12] Taking advantage of this broad capacity for parasitism of the nematode, Glaser undertook its in vitro culture. When first accomplished there was good multiplication for only a few months. Passaging the nematodes through beetle grubs restored the vigor of the strain for further culture. Later it was found that adding to the cultures ground-up beetle grubs, or desiccated, powdered, bovine ovary would prevent the dying out, and permit serial transfer indefinitely.[13]

Due to the interest attached to controlling so obnoxious a pest as the Japanese beetle, Glaser cooperated in a project of the New Jersey Department of Agriculture to establish the parasite in geographical areas of high beetle populations. In this the U. S. Bureau of Entomology later participated. By continued development and modifications of methods of culture[14] the nematodes were eventually grown by the tens of millions weekly under standard conditions, and transferred to soil areas infected with the grubs. It was demonstrable[15] that the nema could thus become established under field conditions. that grub populations were reduced, and that more pests than the Japanese beetle grub were susceptible to being attacked by the parasite. This work constituted the first large-scale use of nematodes in assisting in the control of an agricultural pest, and should be rated one of the striking episodes of the parasitology of recent decades.

As a climax to the study of methods for growing *N. glaseri* on a gross scale, Glaser turned his attention as well to their development under sterile conditions, and succeeded.[16] He thus brought to confinement in sterile media in the laboratory test tube a nematode parasite that can be carried there by routine serial transfers in-

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[9] Glaser and Coria, J. Exper. Med. **51:** 787–806, 1930; J. Parasitol. **20:** 33–37, 1933; Am. J. Hyg. **21:** 111–120, 1935; Am. J. Hyg. **22:** 221–226, 1935; J. Parasitol. **21:** 190–193, 1935; See also Brown, Science **80:** 409–410, 1934.

[10] Science **71:** 16–17. In 1918 Glaser and A. M. Wilcox reported the occurrence of a *Mermis* epidemic amongst grasshoppers (Psyche **25:** 12–15).

[11] J. Washington Acad. Sci. **19:** 436–440, 1929.

[12] State of New Jersey Dept. Agric., Circ. 211, 1–34, 1932.

[13] Science **73:** 614–615, 1931; J. Exper. Zool. **84:** 1–12, 1940.

[14] McCoy and Glaser, State of New Jersey Dept. Agric., Circ. 265, 1–9, 1936; McCoy and Girth, Ibid, Circ. 285, 1–12, 1938.

[15] Glaser and Farrell, J. New York Entom. Soc. **43:** 345–371, 1935; Girth, McCoy and Glaser, State of New Jersey Dept. Agric., Circ. 317, 1–21, 1940; Glaser, McCoy and Girth, J. Parasitol. **26:** 479–495, 1940.

[16] Proc. Soc. Exper. Biol. and Med. **43:** 512–514, 1940.

definitely, with a new generation each week. To this was added another species, *Neoaplectana chresima,* similarly cultivable under sterile conditions.[17]

From the forms parasitizing insects, Glaser's interest was extended to the possibility of growing in vitro some of the nematode parasites of mammals. It was discovered that this could be done under sterile conditions for the free-living stages of *Haemonchus contortus,* and occasional success was had in growing the parasitic stages of this ovine form into the late 4th (2nd parasitic) larval stage. These results,[18] though achieving only partial success measured in terms of the whole life cycle, appear not to have been surpassed for parasitic nematodes in the decade since their announcement.

During the war years Glaser's attention was focussed on special "war" problems. Of one it may be said that he tested the possibility of there being a non-human experimental host for the plasmodia of man, with an ingenuity and persistence worthy the value of such a potentiality, although as with earlier investigators, the problem proved refractory.

Glaser's methods in research were those of the artist and scholar, rather than the "cook" and statistician. He was gifted with originality, an active, facile imagination that continually saw new possibilities of approach. What had not previously been accomplished represented no block against his attack and his published papers rarely belabored the pains with which he assayed experimental ideas and assessed their results. He was always, in essence, the biologist working to isolate and throw light on the character of the factors that participated in the interrelationships existing between living things and the things that existed within them. He set himself few easy tasks.

Rudolf W. Glaser was born and grew up in Baltimore, Md., the son of Dr. Charles Glaser, a consulting chemist. (Dr. Otto C. Glaser, the biologist, is a brother.) His doctorate training was obtained at Harvard (Sc.D., 1914), after an A.B. at Michigan. From 1912 to 1920 he was with the U. S. Bureau of Entomology. Since 1920 he had been at the Rockefeller Institute for Medical Research, and was an associate member at the time of his death. A biographical note in the Princeton "Herald" appropriately referred to him "as one of the best-loved members on the Institute Staff."—NORMAN R. STOLL, *Department of Animal and Plant Pathology, Rockefeller Institute for Medical Research, Princeton, N. J.*

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[17] Glaser, McCoy and Girth, J. Parasitol. **28:** 123–126, 1942.

[18] Glaser and Stoll, Science **87:** 259–260, 1938; Parasitology **30:** 324–332, 1938. A summary by Glaser of "Germ-free culture of certain invertebrates" is the chapter so titled in Micrurgical and Germ-Free Methods, J. A. Reyniers, Editor, C. C. Thomas, Springfield, Ill., 1943.